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PHYSIOLOGY

COMPENSATION OF THE DIGESTIVE FUNCTIONS AFTER PARTIAL RESECTION OF THE STOMACH IN DOGS*

S. I. Filippovich

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(Received April 23, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

The present work presents some results of investigations devoted to the problem of compensatory adaptation in the digestive system which has been studied in recent years by the laboratory team.

I. P. Razenkov's investigations into the features and mechanisms of digestive adaptation to qualitatively different diets [14] as well as to conditions of lowered barometric pressure [15] are well known.

Very valuable data have also been obtained on the question of compensatory functional and structural rearrangement of the digestive system in some pathologic conditions. In particular, clinically important data have been obtained on enhancement of the secretory function of the digestive tract in renal disorders and also on the morphologic and physiologic rearrangement of the gastric mucosa following removal of the pyloric part of the stomach or of the fundus and the body [13]. The latter studies, carried out in I. P. Razenkov's laboratories as early as 1938-1939 (Lazovskii, Sharovatova and Kogan), served as a premise for our elaboration of this question.

The problem of compensatory adaptation of the organism following various systemic lesions has attracted a great deal of attention both in the Soviet Union and abroad. One of the least thoroughly studied aspects of the problem is the mechanism of compensatory processes following "exclusion" of various parts of the gastrointestinal tract. The material available in the literature touches not so much on the mechanisms of compensation as on the characteristics of the changes in the activity of the digestive system following injury or "exclusion" of its parts, in particular resection of the stomach.

In addition to extensive clinical literature (Gordon [1949, 1951, 1957], Shlapoberskii and Neporent [1945], Neporent [1956], Abarbanel [1938, 1950], Shekhter [1946], Neimark [1956], Vitkin [1936], Raiz [1949] etc.) there are also some experimental works dealing with this subject (London et al. [1911-1913], Ivy et al. [1931-1940], Kanshin [1939], Hertel [1930], Everson [1952-1954], Mann, Graham [1932], Seleznev [1955], Petrov [1955] etc.). A number of theoretical questions concerning the dynamics and mechanisms of functional compensation following extensive resection of the stomach still remain unanswered; these include the following: whether compensation occurs as the result of regeneration and restoration of function of the affected organ or as the result of other parts of the system taking over the function; the nature of the inter-relationship of local and central regulatory mechanisms in compensatory reactions; the inter-relationship of functional and structural compensation, etc.

We therefore posed the following problems: 1) to demonstrate concrete forms of compensatory reactions following resection of the stomach in long-term experiments on dogs; 2) to trace the dynamics of compensatory reactions over a long period of time postoperatively, making a study of the degree and order of inclusion of various organs and functions of the digestive system into the process of compensation; 3) to elucidate the mechanisms of the compensatory process and agents which would stimulate it.

* In honor of the 70th birthday of the eminent Soviet physiologist Ivan Petrovich Razenkov the Editors have included in this section of the journal a number of works on the physiology of digestion carried out in recent years by a team of collaborators.

EXPERIMENTAL METHOD

The first results were obtained by us in long-term experiments on 28 operated dogs (with gastric and intestinal fistulas, exteriorized pancreatic duct and isolated loops of the small intestine) 22 of whom had been subjected to gastric resection. Control experiments were carried out on 6 dogs.

The following determinations were made: secretion and enzyme production of gastric glands, pancreas and intestine in response to various stimuli; motor (evacuation and peristalsis) activity of the stomach and intestine, using mechanographic recording and roentgenologic studies. A number of general factors was also determined: changes in body weight in all the dogs, in some dogs the morphologic blood picture, blood sugar and absorption and assimilation of certain substances.

Once the mean normal values had been determined, gastric resection (usually 2/3, in some dogs 4/5) was performed by the Billroth II method as modified by Hofmeister-Finsterer. Two to three weeks postoperatively the same determinations were carried out systematically. The maximal period of follow-up after gastric resection was 2 years.

EXPERIMENTAL RESULTS

The dogs were divided into 3 groups in accordance with their general condition following extensive gastric resection:

Group I – dogs who showed rapid restoration or stabilization of body weight, normal food excitability, absence of signs of disorders of intestinal function, vomiting, etc. This group included 5 dogs.

Group II – dogs who showed periodic loss of weight or prolonged underweight state, periodic disturbances of digestive activity, impairment of food excitability, i. e. signs of partial compensation of the disrupted functions. This group included 12 dogs.

Group III – dogs (5) who died during a period of 1 year (after 5-7-9 months) with progressive loss of weight, cachexia, manifestations of disturbances of intestinal function, anemia. These animals showed marked disturbances of food excitability.

Thus, despite the fact that the given method of operation causes sharp impairment of the digestive function as the result of dropping out of normal gastric function and normal action of pancreatic juice and bile on food, the organism can in the majority of cases mobilize compensatory mechanisms, correcting abnormal conditions created by the operation.

The question arises whether it can be supposed that compensation occurs by virtue of the remaining part of the stomach (part of the fundus and cardia)?

It must be noted that the overwhelming majority of clinical investigations of the contents of the stomach stump show the presence of persistent, prolonged achylia or hypofunction of the gastric glands in patients who had sustained resection of two-thirds of the stomach.

Some experimental data, as well as investigations carried out in our laboratory, confirm the presence of prolonged hyposecretion by the gastric glands after a similar operation in dogs. Moreover, roentgenologic observations on 7 dogs (up to 2 years postoperatively) and autopsies on 5 dogs long after the operation (from 6 to 12 months) showed no appreciable enlargement of the stomach stump. These facts indicate that the stomach stump cannot play an important part in the compensatory process.

Evidently, the compensatory morphologic rearrangement and regeneration of the affected stomach is only possible in cases where only separate parts of the stomach had been removed (Lazovskii, Sharovatova and Kogan, 1939) but not such an extensive portion as was the case in our investigations.

To what extent does the pancreas participate in the process of compensation?

According to the data of I. V. Malkiman and E. A. Pechatnikova, obtained in experiments on dogs with exteriorized pancreatic duct, there is considerable and prolonged lowering of external secretion function of the gland in response to bread and meat. Several months after the operation there is sometimes a tendency to restoration of function followed by another sharp decrease. There is also a substantial change in the total amount of enzymes in the pancreatic juice.*

* See I. V. Malkiman's paper in the same issue.

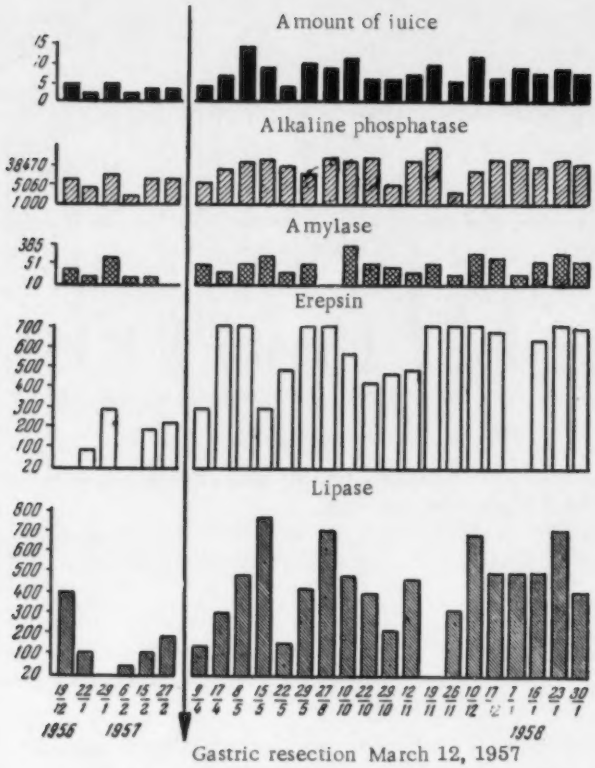

Fig. 1. Secretory function of the small intestine before and after gastric resection in the dog Sulak. Stimulus – irrigation with peptone solution. Enzyme activity is expressed in conventional units.

The work of some foreign authors (Richman, 1954; Annis, Hallenbeck, 1952) also attests to a sharp drop in the secretory activity of the pancreas in dogs following resection of the distal part of the stomach.

These data indicate that the pancreas also cannot play an important role in the compensatory rearrangement of the activity of the digestive system after extensive gastric resection.

Unlike the secretory function of the stomach and the pancreas, the secretory activity of the small intestine after gastric resection is, according to our data, not only not depressed but in most cases enhanced.

Comparison of "periodic" intestinal secretion with secretion obtained in response to mechanical stimulation and to a chemical stimulus (irrigation of the intestine with peptone solution) showed that increased secretion was more marked in response to the chemical stimulus.

Enhancement of enzyme-secreting function is particularly noticeable.*

Increased concentration and total amount of enzymes were observed beginning with the 1st-2nd month after operation and persisted, sometimes with some fluctuations, throughout the period of investigations (up to $1\frac{1}{2}$ years after gastric resection). Particularly marked was the secretion of enzymes participating in protein breakdown, and in isolated cases of amylase and lipase also (Fig. 1).

With respect to the dynamics of changes in the motor-evacuation activity of the digestive tract following

* The following enzymes were determined: enterokinase, alkaline phosphatase, amylase, lipase, erepsin; the methods used were those developed or modified in G. K. Shlygin's laboratory (Shlygin, 1950; Fomina, Mikhlin and Shlygin, 1952).

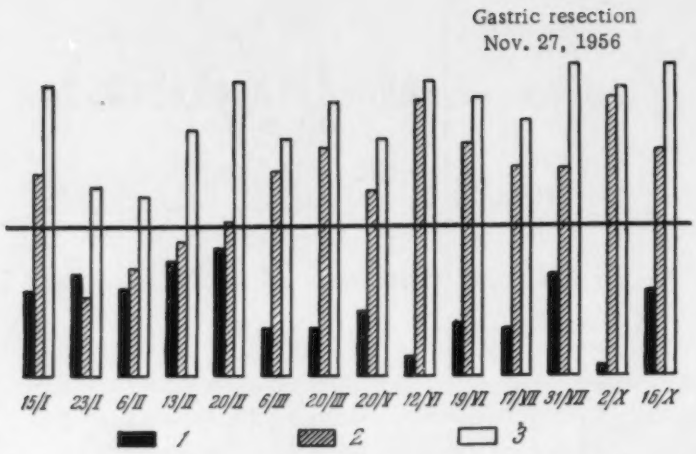

Fig. 2. Evacuation function of the stomach and small intestine after gastric resection in the dog Belianka.
1) Time over which the milk mixture remained in the stomach; 2) time during which the milk mixture remained in the small intestine; 3) total time.

gastric resection, the experiments showed that as a rule there was disturbance of periodic "fasting" motor activity of the small intestine expressed in increased periods of work or even continuous contractions. Restoration of periodicity was observed after an average of 2-3 months.

Starting roughly about the same time, some dogs began to show, as demonstrated by roentgenologic examination of gastric and intestinal evacuation, slowing down of the passage of food along the small intestine. The slowing down increased gradually and reached a maximum a long time after operation (6-12 months). It is interesting to note that this slowing down is more pronounced when food is evacuated from the stomach stump more quickly (Fig. 2). There is a simultaneous slowing down of peristalsis in the small intestine.

The appearance of the features described above a long time after the operation suggests that these processes are associated with morphologic rearrangement of the digestive tract.

The compensatory character of increased secretory function of the small intestine and change in the transport of food is indicated by the fact that the degree and effectivity of these phenomena following on gastric resection are closely connected with the dynamics of change in some general indices of the dog's condition, in particular body weight.

The first results of the work thus showed that compensation of impaired digestive function in this case occurred by virtue of rearrangement of the secretory-motor activity of the small intestine. Further investigations were devoted to the question of the mechanisms and means of achievement of compensatory reactions.

With this aim in view, experiments were performed on dogs with fistulas of two isolated segments of the small intestine. In one of the segments the neurovascular connection with the mesentery was preserved; in the second segment the innervation was interrupted. The experiments showed that there was increased secretion in the innervated intestinal segment postoperatively whereas in the denervated segment such increase was either absent or was only very slight (Fig. 3). These facts speak of the significance of extramural nervous pathways in the compensatory-adaptational reaction of the digestive system.

It is interesting that increased secretory function of the small intestine was most marked when products of food digestion (peptone) were used as stimuli. It may be supposed that one of the quickly mobilized compensatory mechanisms may be a change in the sensitivity of small intestine receptors consequent on changes in the physicochemical properties of food (chyme) which gains access to the small intestine following gastric resection, with resulting rearrangement of unconditioned reflex activity. In the light of the work of V. N. Chernigovskii and V. A. Lebedeva (1947-1951) on the mechanisms of intestinal chemoreception, such a hypothesis is quite

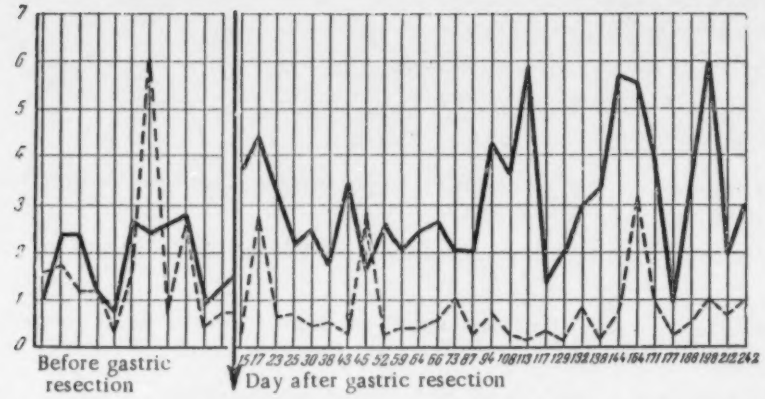

Fig. 3. Secretion of juice on mechanical stimulation of isolated segments of the small intestine in the dog Belianka.
——— secretion from the innervated segment, ------ secretion from the denervated segment.

feasible. Our next problem is that of studying the role of the properties of chyme in the effectiveness of the observed compensatory reactions.

It must also be emphasized that the state of the food center also plays a very important part in the course of the compensatory process, in some cases this role being decisive. The majority of our experimental dogs showed, after gastric resection, various degrees of food excitability, especially the group III dogs who died several months postoperatively. The next problem in this connection is elucidation of the causes and mechanisms responsible for the disturbances of the functional state of the food center under these conditions.

According to preliminary data, these disturbances are connected both with the exclusion of so vast a receptor area as the gastric mucosa and with impairment of absorption from the digestive tract with consequent changes in blood chemistry.

The results reported are a confirmation and further development of the theoretical views of I. P. Razenkov who has always stressed the significance of the initial functional state of the digestive organs and their higher regulatory mechanisms in the reactivity of the digestive tract.

SUMMARY

This article presents results of examination of the compensatory processes and the function of the digestive system after partial (two-thirds and four-fifths) resection of the stomach in dogs. These data were obtained in experiments on 28 dogs, observed up to 2 years after the operation. The secretory function and the enzyme secretion of the stomach, pancreas and small intestine, as well as the motor function and absorptive property of the small intestine, were studied.

The experiments demonstrated that compensation of the functions of the digestive system took place mainly by way of rearrangement of the secretory motor function of the small intestine. Certain data concerning the mechanisms of compensatory reactions were also obtained.

The role of the extramural intestinal innervation and the functional condition of the food center in compensatory processes was studied in particular.

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DATA CONCERNING ABSORPTIVE PROPERTIES OF THE GALL BLADDER

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Much of the work carried out in the laboratories directed by I. P. Razenkov has been concerned with elucidation of the role of the digestive tract in intermediate metabolism [8]. To this group belong also our studies on the circulation of bile phosphorus compounds in the organism.

In a series of works published earlier we reported the results of studies on the rate of formation of organic phosphorus compounds in the liver and their secretion in the bile. Isotope technique has revealed that 2 hours after administration of radioactive sodium phosphate to a dog per os up to 30% of P^{32} found in the bile is associated with organic compounds, the percentage rising to 85 after 6 hours and to 99% of all the bile P^{32} after 24 hours. Chemical methods and radiochromatography of bile and products of hydrolysis of the organic phosphorus compound extracted from bile, and other methods have shown that almost all the bile phosphorus (up to 99%) is secreted as phospholipid of the type of lecithin.

The possibility of obtaining labeled bile phosphorus compounds in the body by means of biosynthesis allowed us to study the circulation of organic phosphorus compounds of the bile on introduction of whole bile or phospholipids extracted from it into the digestive tract.

Our experiments furnished an answer to two questions: 1) whether organic phosphorus compounds were absorbed in the digestive tract as such or whether inorganic phosphorus was split off from them and 2) whether there was direct passage of absorbed phospholipids into the composition of newly secreted bile.

It was found that phospholipids secreted with bile into the intestine could be absorbed without splitting off inorganic phosphorus.

When dogs are given strongly radioactive or inactive bile by mouth with milk, there is increase in bile formation by the liver and a rise (compared to the control) of the absolute phosphorus content of secreted bile, but this is not accompanied by increase or "dilution" of radioactivity: the specific activity of secreted bile remains unchanged. It follows from this that the phospholipids absorbed from the intestine from the bile introduced into it from a donor dog do not pass directly into the bile of the recipient dog [2, 4, 5].

Having established that bile phospholipids could be absorbed in the intestine without splitting, it was decided to check whether there was also absorption of phospholipids in the gall bladder where, as is known, considerable changes in the composition of the bile with respect to its individual components take place.

There are only scanty and conflicting references in the literature concerning the absorption through the gall bladder wall of various components of bile. It could be supposed that the isotope technique would furnish considerable help in the study of the absorptive capacity of the gall bladder wall [5].

EXPERIMENTAL METHOD

Radioactive bile was collected from the donor dog and introduced in amounts of 5-10 ml into the gallbladder of the recipient dog after preliminary ligation of the bile duct and aspiration of the bile from the

TABLE 1

Protocols of Experiments on Absorption of Labeled Bile Phospholipids from the Gall-Bladder in Dog (Short-term Experiments)

Date of experiment	Dog's weight (in g)	Radioactive bile introduced into the gall bladder (in ml)	Total activity of introduced bile (in imp/min)	Residual activity in the gall bladder at end of experiment (in imp/min)	Remarks
15/I 1954	8 500	7,0	$2,3 \cdot 10^6$	$2,3 \times 10^6$	No radioactivity was detected in the liver and blood serum in these expts.
1/IV 1954	11 000	7,5	$6,86 \times 10^5$	$6,85 \times 10^5$	
12/V 1954	6 000	5,0	$7,2 \times 10^5$	$7,18 \times 10^5$	

gall-bladder. In long-term experiments the bile was introduced into the gall bladder through a fistula, in amounts of 5-8 ml. In order to avoid increased pressure in the gall bladder the radioactive bile in the latter case was introduced in 3-4 divided doses with intervals of 60-90 minutes following preliminary evacuation of the gall bladder. Only recently operated dogs were taken for these experiments. As reported by us earlier [6] dogs with gall bladder fistulas often develop, in time, degeneration of the gall bladder mucosa and morphologic changes in the liver.

The total activity of the introduced bile was from 800,000 to 2,000,000 imp/minute. Blood was taken from the femoral vein, in long-term experiments from the saphenous vein, 5, 10, 15, 30, 60, 90, 120, 180, 240, 300 and 360 minutes after introduction of radioactive bile. Serum was examined for total, inorganic and lipoid phosphorus and their radioactivity.

At the end of the experiment radioactivity of the liver was determined. Moreover the gall bladder was excized and placed into a crystallizer with water for 30-40 hours. During this period the water was changed 2-3 times. The summated radioactivity of the washings and the residual radioactivity in the gall bladder wall were determined. The percentage of P^{32} absorbed during the entire experiment was calculated from data on the amount of administered P^{32} and that remaining in the gall bladder at the end of the experiment.

This series included 4 short-term experiments and 4 long-term experiments, the latter on dogs with gall-bladder fistulas. The duration of each experiment was from 5 to 8 hours.

EXPERIMENTAL RESULTS

There was no passage into the blood of labeled bile phospholipids or inorganic radioactive phosphorus split off from them. Nor was any radioactivity detected in the liver (Table 1).

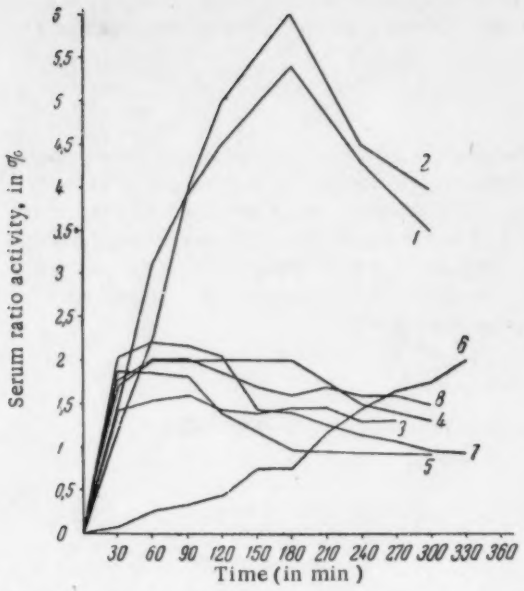
In the second series of experiments radioactive sodium phosphate ($Na_2HP^{32}O_4$) was introduced into the gall bladder and the passage of radioactive phosphorus into the blood traced in time.

Since the content of total, inorganic and lipoid phosphorus during the experiment usually showed only slight fluctuations, the calculation of blood serum radioactivity was carried out as follows: the number of impulses per minute of 1 ml serum was multiplied by one twentieth of the animal's weight in grams, taking this last value arbitrarily as the weight of the total amount of blood serum circulating in the given animal.

Thus the amount of P^{32} present in the blood serum at the moment of examination could be determined as percentage of the total amount of P^{32} introduced into the gall bladder.

In order to find out the significance of the parasympathetic nervous system in the processes of absorption in the gall bladder, atropine in the dose of 0.05 mg per 1 kg body weight was given subcutaneously in some of the experiments; the injection was made 30 minutes prior to administration of P^{32}. In other experiments the gall-bladder receptors were excluded by irrigating the bladder wall, 30 minutes prior to administration of P^{32}, with 2% solution of dicaine (1-2 ml). The latter was aspirated from the gall bladder before the P^{32} was introduced into it.

There is a number of references to the action of dicaine on the peripheral and central parts of the nervous system [1, 3, 4, etc.]. The role of the nervous system in the processes of absorption in the digestive tract and in processes of permeability of glandular tissue has received much attention.



Blood serum radioactivity following administration of $Na_2HP^{32}O_4$ into the gall bladder in dog (short-term experiments).
Curves 1 and 2 – control experiments; curves 3, 4, 5 – against the background of the action of preliminarily administered atropine; curves 6, 7, 8 – against the background of the action of preliminarily administered dicaine.

M. Lindeman [6], working in I. T. Kurtsin's laboratory, investigated the effect of changes in the functional state of the cerebral cortex on absorption of water and sodium chloride from the gall bladder.

The results of our studies on the absorption of radioactive sodium phosphate in the gall bladder in control experiments and with administration of dicaine and atropine are presented in the figure.

In the control experiments slight blood serum radioactivity was observed 3 hours after the beginning of the experiment (curves 1, 2). All the activity was present in the inorganic phosphorus fraction of the blood serum. No activity was found in the lipoid fraction of the serum.

In this way it was possible to trace, in relative units, the dynamics of absorption. In addition to this, the total amount of absorbed P^{32} was found from the difference between the introduced and residual P^{32} in the gall bladder at the end of the experiment using the method indicated above (Table 2). In these experiments about 80% of the introduced P^{32} was absorbed in 5 hours. Injection of atropine 30 minutes before the introduction of P^{32} caused sharp inhibition of P^{32} absorption from the gall bladder. In this case the curve for absorption of inorganic phosphorus differed markedly from the control (curves 3, 4, 5). Only 10-20% of the introduced P^{32} was absorbed from the gall-bladder during 5 hours. Inhibition of P^{32} absorption from the gall bladder was also observed after irrigation of its walls with dicaine (see Figure, Curves 6, 7, 8). In the experiment represented by curve 6 only part of the dicaine introduced into the gall bladder could be aspirated and in this case the inhibition of P^{32} absorption was the greatest.

The experiments carried out thus indicate that phospholipids are not absorbed in the gall bladder, whereas inorganic phosphate is absorbed rapidly. In the course of 5 hours up to 80% of phosphate introduced into the

TABLE 2

Protocols of Experiments on Radioactive Sodium Phosphate ($Na_2HP^{32}O_4$) Absorption from the Gall Bladder in Dog

Date	Dog's weight (in g)	P^{32} introduced (in imp/ min)	Residual activity in the gall bladder at end of experiment (in imp/ min)	Amount of P^{32} absorbed from gall bladder as % of P^{32} introduced
	Control experiments			
2/III 1954	14 300	1.43×10^6	$2.86\times10_6$	80
16/III 1954	7 500	9.4×10^6	2.02×10^6	73
	Experiments with administration of atropine			
12/III 1954	13 350	1.35×10^7	1.24×10^7	8
18/IV 1955	13 750	1.37×10^7	1.21×10^7	12

gall bladder is absorbed. Injection of atropine or irrigation of the gall bladder walls with dicaine prior to introduction of phosphate into it causes sharp inhibition of absorption: in 5 hours only 10-20% of introduced phosphase is absorbed in these cases. This points to the importance of the parasympathetic nervous system and the receptors of the gall bladder mucosa in the process of absorption.

SUMMARY

These experiments were devoted to the study of the absorption of labeled P^{32} phospholipids and inorganic sodium phosphate from the bile duct of a dog. Labeled phospholipids were obtained by biosynthesis in the body of a donor dog. Phospholipids introduced into the recipient dog's gall bladder were present in the bile collected from the donor dog. The absorption from the bile duct was judged by the appearance of P^{32} in the blood serum and by the activity retained in the gall bladder. The effect of subcutaneous injection of atropine (in the dose of 0.05 mg per kilogram of body weight) and administration of 2% solution of dicaine into the gall bladder on the absorption of inorganic phosphate was also studied in short-term experiments.

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THE ROLE OF PRODUCTS OF FOOD DIGESTION IN THE STIMULATION OF PANCREATIC SECRETION

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In his first independent experimental work on digestion, which was devoted to investigation of the mechanism of the second phase of gastric secretion, I. P. Razenkov posed the question concerning the role of the products of peptic digestion in the stimulation of the secretory activity of gastric glands. Further investigations in this direction carried out by I. P. Razenkov and his collaborators led him to conclude that the chemical stimuli in the form of foodstuffs and products of their digestion, especially products of protein digestion, were able not only to stimulate gastric secretion but were also a powerful factor affecting the activity of various organs and tissues, among them the digestive organs. I. P. Razenkov ascribed great significance to the role of humoral factors in the process of adaptation of the body to its environment and correlation of function between organs, and has repeatedly stressed that they act most frequently through the nervous system. This was particularly clearly formulated by him on interpretation of the change in the ratio of the magnitude of the first and second phase of gastric secretion when the animals were kept on a prolonged bread or meat diet.

The work of I. P. Razenkov which is most closely related to the present study is that on the mechanism of secretin formation. The experiments carried out by I. P. Razenkov et al. [2, 3] showed that peptone, gastric juice and products of digestion of fibrin, meat and milk played a substantial part in the mechanism of secretin formation in the duodenum. However, whether the products of peptic digestion of proteins participate directly in the mechanism of excitation of the intestinal phase of pancreatic secretion has not been definitely established up to the present time. This question has not been touched on in the investigations of I. P. Razenkov and his team of workers, nor has it been studied in detail by other investigators. A small number of experiments devoted to this question has shown that peptone, egg albumin, meat broth introduced into the stomach through a tube "do not exert a marked effect on the secretion of pancreatic juice and in most cases are either equivalent to water or even inferior to it with regard to stimulating secretion" (Dolinskii [1]).

On the other hand Thomas and Crider assert that the products of protein digestion, introduced directly into the intestine, exert a stimulating effect on the external secretion of the pancreas.

The literature data cited are not only conflicting but are also insufficiently convincing and cannot serve as proof of the stimulating action of the products of digestion on juice secretion, since in both cases the experiments were performed on dogs with normally functioning stomach, and the possibility of the introduced agents' stimulation of the pancreas being secondary to secretion of acid gastric juice was not excluded. Yet the question as to whether the products of peptic digestion can stimulate pancreatic juice secretion directly is of great significance both theoretically and in medical practise, since gastric resection and even total gastrectomy have become an extensively used surgical procedure.

The aim of our experiments has been the investigation of the changes occurring in the secretory activity of the pancreas as the result of gastric resection.

EXPERIMENTAL METHOD

The investigations were carried out on three dogs with exteriorized pancreatic ducts. The dogs were kept on a vegetable-milk diet and received daily from 2 to 3 g soda. The experiments were staged 18 hours after the last meal. The juice was collected by the Delezen method. The amount of juice secreted was noted every 15 minutes. Prior to administration of the stimulus the juice was collected over a period of one hour (arbitrary secretion according to Dolinskii) which served to establish the functional state of the secretory apparatus on any given day of experiment. The secretion norm was established to 100 g white bread, 100 g meat and 200 ml milk. The amount of juice and concentration of enzymes (amylase, trypsin and lipase) were determined in 1 ml of juice. Once the secretion norm was established the dogs were subjected to gastric resection by the Billroth II method in Hofmeister-Finsterer modification. From two-thirds to four-fifths of the stomach was resected. The period of observation lasted from 2 to 18 months.

EXPERIMENTAL RESULTS

The data obtained revealed that resection of the distal part of the stomach led to significant changes in the secretory activity of the pancreas, the degree and direction of these changes being related both to the kind of food stimuli employed and the time which had elapsed since operation. Thus, for example, the volume of secretion in response to bread used as a stimulus was markedly lowered for a period of about 4 months postoperatively, the decrease amounting to an average of 55-60%. The amount of juice then begins to increase reaching on some experimental days the initial values. However, this period is transient (about 3 months) and a more pronounced and persistent decrease of secretion in response to the given stimulus then supervenes. During this last period, which continues for over 10 months, the amount of juice is on average 15-25% less than the initial values before resection.

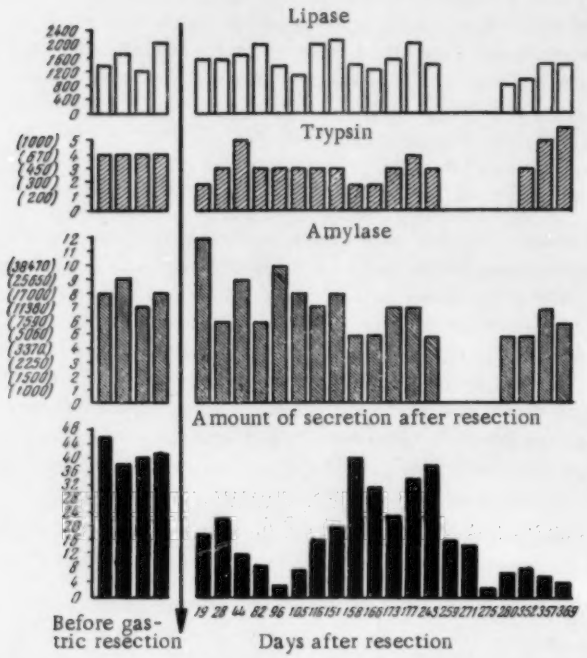

Fig. 1. Pancreatic juice and enzyme secretion by the pancreas in the dog Khudoi. Stimulus – Bread 100 g.
The amount of pancreatic juice is represented by black columns; amylase concentration by columns hatched from left to right; trypsin by columns hatched from right to left; lipase by white columns; ↓ moment of gastric resection. At left along the ordinate are arbitrary units expressing the amount of enzyme in 1 ml juice.

TABLE 1

Pancreatic Secretory Function in the Dog Lata (food stimulus milk 200 ml)

	Before gastric resection																													
Amount of pancreatic juice during expt (in ml)	23,0		21,0		28,0		22,0		29,0		20,0		23,0		23,0		22,0													
Amylase in arbitrary units	57 700		57 700		57 700		86 550		—		57 700		—		1 700		—													
	After gastric resection																													
Amount of pancreatic juice during expt (in ml)	34,0		47,0		34,0		32,0		20,0		80,0		42,0		27,0		44,0		18,0		54,0		54,0		50,0		23,0		22,0		15,0		15,0		27,0		36,0	
Amylase in arbitrary units	7 590	14	11 380	15	7 590	16	11 380	24	11 380	28	7 590	30	11 380	31	17 000	32	25 650	35	11 380	38	7 590	42	11 380	44	17 000	45	38 470	49	1 700	57	1 700	60	38 470	66	38 470	71	5 060	77
Days after resection																														

As regards the secretion of enzymes, amylase, trypsin and lipase, their concentration in the juice in most cases is in reverse relationship with the amount of juice secreted in unit time, i. e. as the rate of juice secretion drops there is a corresponding increase in unit time of concentration of the enzymes.

The following data are furnished as illustration (Fig. 1).

Quite a different picture is observed when milk is used as a stimulating agent. Since the data obtained on all three animals are basically identical, only the results obtained on one of them, the dog Lata, are given (Table 1).

Analysis of the material contained in the table indicates that pancreatic secretion in response to milk not only does not drop after resection but, with the exception of isolated experiments, even rises. Amylase concentration (the other enzymes were not determined in this dog) is lowered in isolated experiments which may be explained by increased hourly rate of juice secretion.

Juice secretion in response to meat, as that in response to bread, is lowered but not to the same degree in all the dogs. As in the case of bread and milk, the enzyme activity is related to the hourly rate of juice secretion.

It must be noted that the enzyme concentration, or enzyme activity, is not a reliable criterion for evaluation of the secretory activity of the pancreas unless the hourly rate of juice secretion is taken into account. The concept of enzyme-secretion, i. e. given concentration of enzyme multiplied by the hourly volume of juice secretion proposed by some workers is more meaningful. From this point of view factual material indicates that resection of the distal part of the stomach leads to marked lowering of the secretory activity (juice and enzyme) of the pancreas in response to bread and meat stimuli even in those cases in which the enzyme concentration is not decreased but rather somewhat increased, since the hourly volume of juice secretion is substantially decreased. The secretory activity of the pancreas is, on the contrary, increased in response to milk at least over a period of several months after gastric resection, although the concentration of amylase on isolated experimental days is somewhat diminished.

The data on the effect of milk on pancreatic secretory activity posed the question whether the facts observed in connection with decreased secretion in response to other stimuli (bread, meat) were the result of reduction of the functional possibilities of the pancreas itself as the consequence of transection during gastric resection of both vagi passing through the pylorus and duodenum to the pancreas, or whether the diminution of secretion in response to these stimuli depended on other causes.

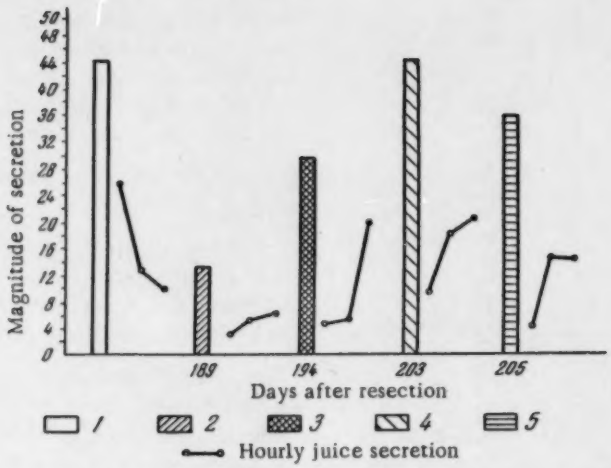

Fig. 2. Curves of pancreatic juice secretion before and after gastric resection.
1) Amount of juice in response to meat before gastric resection; 2) amount of juice in response to meat after resection; 3) amount of juice in response to meat + 30 ml gastric juice; 4) amount of juice in response to meat + 70 ml gastric juice following 18-hour digestion in thermostat; 5) amount of juice in response to meat + 70 ml gastric juice.

With this aim in view we undertook a series of experiments with meat and gastric juice added to it, as well as with products of peptic digestion of meat by gastric juice in a thermostat and their subsequent neutralization. The data obtained are presented in Figure 2. These indicate that before gastric resection the pancreatic juice secretion was 44 ml (typical experiment before resection), while on the 189th day after resection it was equal to only 14 ml. However, juice secretion in response to 100 g meat after its peptic digestion by gastric juice in a thermostat was the same as prior to resection.

In the experiment in which 100 g meat and 70 ml gastric juice were used the secretion of juice was increased but did not reach the initial value obtaining prior to resection. Attention is attracted by the character of the juice secretion curve. Before resection around 50 and over 50% of juice is secreted during the first hour, with a subsequent decline. After resection the situation is reversed: maximal amount of juice is secreted during the 3rd hour. This suggests that juice secretion is at this time effected chiefly by means of the intestinal phase of pancreatic secretion, i. e. as the result of entry of the products of digestion into the jejunum. The following experiments were performed by us in order to discover whether the products of protein digestion could have a primary stimulating effect (without the mediation of evoked gastric juice secretion) on pancreatic secretion after gastric resection by the Billroth II method, when they pass not into the duodenum but into the jejunum. A year and more after gastric resection a stomach stump fistula was made in one of the dogs in order to make it possible to control the reaction of its contents and to introduce food and other stimuli not per os, but directly into the cavity of the remaining part of the stomach.

The data obtained revealed that a 12% solution of peptone was able to evoke relatively large pancreatic secretion (Fig. 3). Figure 3 shows that 200 ml 12% peptone solution introduced into the cavity of the stomach stump evokes pancreatic juice secretion which is more than twice that evoked by a similar amount of water.

The question concerning the mechanism of pancreatic secretion in response to the introduction of HCl into the duodenum has not so far been clarified. It is all the more interesting that we have discovered that atropine (1.5-4 mg) given to the dog subcutaneously 15-45 minutes prior to administration of peptone produced a sharp decrease in the volume of pancreatic juice secretion elicited by the latter, with practically no change in enzyme activity, although the rate of juice secretion was considerably lowered and it might have been expected

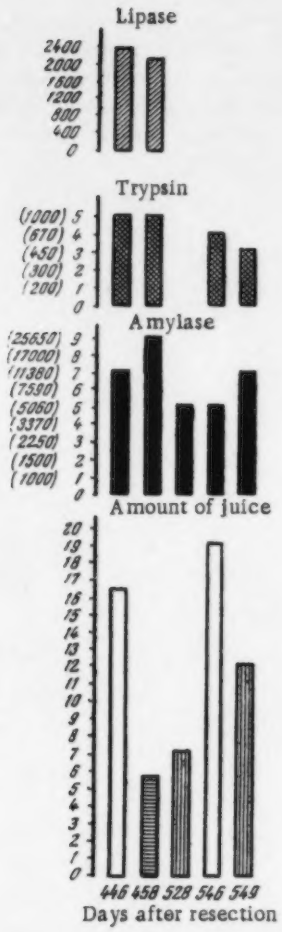

Fig. 3. Pancreatic secretion in response to peptone solution after gastric resection.
White column – amount of juice secreted in response to stimulation with 12% peptone solution; horizontally hatched column – amount of juice secreted in response to water; columns with vertical hatching – amount of juice secreted in response to peptone solution after injection of atropine. Enzyme concentration is shown at the top of the figure. On the left along the ordinate are arbitrary units expressing the amount of enzymes in 1 ml juice.

that there would be some increase in the concentration of enzymes in the jejunum.

It is difficult to postulate the mechanism for this phenomenon without further investigations, and particularly difficult when it is taken into account that under our experimental conditions the peptone passed not into the duodenum, with which pancreatic innervation is closely linked, but into the jejunum.

The factual material obtained leads to the conclusion that resection of the distal part of the stomach in dogs produces considerable changes in the secretory activity of the pancreas, their degree and direction being dependent both on the food stimuli used and on the time elapsed postoperatively.

The magnitude of juice secretion in response to bread is decreased by 55-60% for 3-4 months, then begins to increase reaching, on isolated experimental days, the initial values observed prior to resection. After 2-3 months a period of more drastic and persistent lowering of secretion sets in. During this period, already lasting for over 10 months, juice secretion amounts to 15-25% of the initial values. Enzyme concentration is near normal, and on isolated experimental days above it.

The magnitude of juice secretion in response to meat is lowered but not to the same extent in all the dogs. The amylase concentration is somewhat increased. The concentration of other enzymes fluctuates within normal limits.

Pancreatic juice secretion in response to milk is not only not decreased but is even either increased or within normal limits following gastric resection. The amylase concentration is slightly lowered and on isolated experimental days near to normal.

Addition of gastric juice to meat, as well as of products of peptic digestion of meat in a thermostat, elicit juice secretion which is near to normal, indicating that gastric resection does not, basically, diminish the functional capacities of the secretory apparatus of the pancreas.

Peptone solution introduced into the cavity of the stomach stump through a fistula elicits pancreatic juice secretion which is more than twice that elicited by the same amount of water.

Atropine inhibits the secretory activity of the pancreas elicited by peptone, decreasing the amount of juice secreted despite the fact that the concentration of enzymes is not altered substantially. All these data point to participation of a nervous mechanism in this process.

SUMMARY

This investigation was carried out on dogs in whom the pancreatic duct was exteriorized by surgical operation. The quantity of the juice and the concentration of the enzymes (amylase, trypsin and lipase) secreted in response to different stimuli were determined. After establishing the normal values for the secretory function of the pancreas two-thirds to four-fifths of the stomach was resected in these dogs. The latter were observed for 18 months.

The obtained data demonstrate that stomach resection causes considerable changes in the secretory function of the pancreas, the degree and the direction of which depended on the food stimuli, as well as on the period which elapsed after the resection. Peptone solution introduced into the cavity of the stomach stump through a fistula provoked pancreatic secretion, the quantity of which exceeded that caused by the same quantity of water by $2\frac{1}{2}$ times. Atropine is able to depress pancreatic secretion, decreasing its quantity, although there is no significant change in the concentration of the enzymes. These data demonstrate the participation of a nervous mechanism in this process.

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THE ABSORPTIVE FUNCTION OF THE SMALL INTESTINE AFTER SUBTOTAL RESECTION OF THE STOMACH

COMMUNICATION I. CHANGES IN THE ABSORPTION OF GLUCOSE AND WATER BY THE SMALL INTESTINE AFTER PARTIAL RESECTION OF THE STOMACH

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This article is part of a complex study being conducted at this laboratory on the problem of compensating functional disturbances of the digestive system caused by various injuries to the latter, particularly by the resection of various portions of the digestive apparatus [1, 5, 12].

Our task was to study the effect of partial resection of the stomach on the absorptive function of the small intestine in dogs. Despite its great importance to theoretical and practical medicine, this question has been inadequately investigated.

The existing research on this problem for the most part concerns the effect of stomach resection on the assimilation of food substances; the authors judge the absorptive powers of the small intestine according to the assimilability of these food substances [6, 13, 14].

EXPERIMENTAL METHODS

We studied the dynamics of the disturbance and restoration of the absorptive power of the small intestine with subtotal resection of the stomach in chronic experiments on 5 dogs. The diet and general maintenance conditions were the same for all the experimental animals. The dogs were given full-value food in pulverized form at the same times every day 2-3 times per 24 hours. The appetite and general condition were systematically observed, and the dogs were weighed once or twice a week.

The operation isolating an intestinal loop and extirpating the stomach (Finsterer's modification of Billroth's operation No. 2) was performed on the experimental dogs by N. Sh. Amirov.

The effect of stomach resection upon absorption was studied by two different methods. The dynamics of normal glucose absorption and of glucose absorption at different intervals after the resection was studied on two dogs (Naidenysh and Laima) by introducing glucose into the digestive tract (in a dose of 2 g per 1 kg of animal weight) and determining the dynamics of its concentration in the blood. Blood was taken from the vena saphena parva on an empty stomach and then 15, 30, 45, 60, 90 and 120 minutes after ingestion of the glucose. The blood sugar was determined by the Hagedorn-Jensen method.

The absorptive function of the small intestine was studied on an empty stomach on four dogs (Laima, Trezor, Lokhmach, Sulak) with sections of the upper portion of the small intestine isolated according to Thiry and Thiry-Vella. Water and 0.5-1% solutions of glucose were used as the experimental substances. Fifteen ml of the experimental solution was introduced for a period of 7 minutes into the isolated intestinal loop (15-20 cm). The temperature of the experimental substance and of the water used for washing the intestine was constant at 37°.

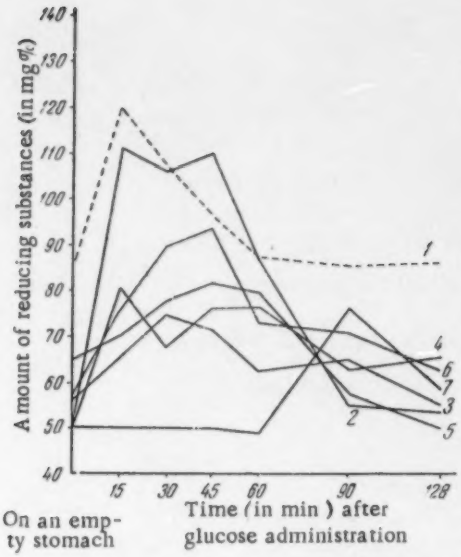

Fig. 1. Glycemic curves of the dog Naidenysh, normally and after resection of the stomach (experiment from May 8, 1956 to March 24, 1958).

1) norm; 2, 3, 4, 5, 6, 7) at different intervals after resection of the stomach (experiment begun May 8, 1956). Ordinate axis represents sugar, in mg%; abscissa axis represents time, in minutes.

The glucose content of the experimental fluids was also determined by the Hagedorn-Jensen method. The amount of absorption was determined from the amount of experimental substance absorbed from the isolated intestinal loop during the 7-minute period and was expressed in percent of the amount of substance administered. By comparing the amount of glucose and water absorption and character of the variations observed in these indices at various intervals after resection of the stomach with the fluctuations in absorption observed in the background experiments, we could estimate the changes in the absorption of these substances caused by resection of the stomach.

EXPERIMENTAL RESULTS

The first of the methods we used to study the character of glucose absorption after resection of the stomach showed that the glycemic curve and the blood sugar level changed considerably after the resection, but these changes were not the same in the two dogs. In Naidenysh, for example, there were more acute disturbances in the blood sugar content and in the character of the glycemic curve. After resection and on an empty stomach, the blood sugar level had decreased sharply. The normal blood sugar in this dog being 80-90 mg%; after the resection, the content decreased during the first month to 50 mg% and remained at 50-60 mg% for 7 months. One to one and a half years after resection, the blood sugar as determined on an empty stomach rose to 90-95 mg%, then decreased sharply again to 50 mg%. Sugar-loading after resection caused, in a large mjaority of cases, a slower increase of the curve to the maximum. A year and 10 months after the resection, glucose was only observed in the blood 90 minutes after its per os administration, the glucose content increasing to 77 mg% (Fig. 1, curve 7).

In the dog Laima, the glycemic curve changed after resection of the stomach, but not in the same way as in the dog Naidenysh. The normal blood sugar content on an empty stomach was 70-80 mg% in Laima; the blood sugar level did not fall after resection of the stomach, but remained at the original level for 1 year and 5 months. Even after sugar-loading, Laima's glycemic curve remained relatively high and was not as pathologic in character as that of Naidenysh (Fig. 2).

Resection of the stomach was well tolerated by the two dogs, Naidenysh and Laima. However, the first week after the operation, Naidenysh sustained a weight loss of 3300 g and another of 1200 g during the second week. Although Naidenysh's general condition was satisfactory, great fluctuations in weight and food excitability were subsequently observed in this dog. During the 23-month observation period, he lost 9350 g, or 42% of his original weight. No sharp decrease in weight was observed in Laima. The dog lost 2 kg during the first two months after the resection, but thereafter none of the sharp variations in weight and food excitability which were observed in Naidenysh were observed in Laima. During the 17-month observation period, Laima lost 3 g, or 19% of her original weight.

According to the well-documented literary data, considerable variations in the blood sugar level and in the character of the glycemic curve are known to take place after resection of the stomach.

It is known that, after resection of the stomach, when food enters directly into the jejunum not only carbohydrate absorption is disturbed, but also the metabolic functions of the liver, especially the glycogenic. It is therefore impossible to conclusively determine the absorptive ability of the small intestine according to the sugar level in the peripheral blood. Therefore, we went on to study absorption in isolated loops of the jejunum.

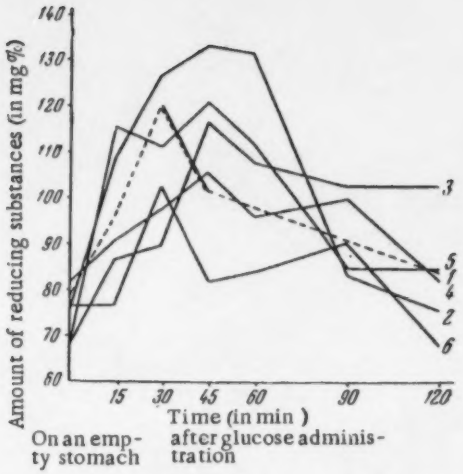

Fig. 2. Glycemic curves of the dog Laima, normally and after resection of the stomach (experiment from October 16, 1956 to March 26, 1958). 1) norm; 2, 3, 4, 5, 6) at different intervals after resection of the stomach (October 16, 1956).

Many investigations [2, 3, 4, 7, 8, 9, 10, 11] have demonstrated the possibility of studying the absorptive function of the small intestine normally and under various pathologic conditions on isolated sections of the jejunum.

Data illustrating the effect of stomach resection on glucose and water absorption by an isolated intestinal section is given in Figure 3.

As Figure 3 shows, stomach resection caused, in the dog Laima, the degree of glucose absorption to decrease during the first month to 25-30% (original level – 50-60%); this index then increased to 60-70% and thereafter remained within normal limits. In this dog, as Figure 3 shows, resection of the stomach increased the absorption of water to 40% during the first month (original level – 20-30%); water absorption then remained high for a long time, 75-85% with occasional drops to 40%.

From Figure 3, one can see that in Trezor (II), glucose absorption was 60-80% before resection. Glucose absorption was within normal limits on the 13th day after resection (75%). Three weeks after the operation, it decreased to 30-50%. Then it became almost normal for a month. Normal water absorption in Trezor was 15-20% (Fig. 3). On the 17th day after the operation, water absorption had increased to 35-45%; it then remained at this level.

Glucose absorption was 50-70% in the dog Lokhmach before the operation. This index decreased to 25-30% for two months after the resection, then increased and settled within the normal range.

Normal water absorption in Lokhmach was 25-40%. This increased to 60-65% during the first month after the resection and then to 65-75%, where it remained for four months. Subsequently, it decreased to 40-50%, approximating the original level.

In the dog Sulak, glucose and water absorption remained low both before and after resection of the stomach, especially after the operation. The normal glucose absorption in this dog was 10-20%; it decreased to 5-7% after resection of the stomach and remained at this level for two months, after which it rose to 15%. Water absorption in Sulak was 10-20% before the operation. One month and 16 days after the resection, it increased to 20-35%, where it remained for 7 months. After this, sharp variations in the amount of water absorption were observed – it would increase to 60%, decrease to 8% and then rise again to 30-35%. It should be mentioned that Sulak sustained a weight loss of 4 kg during the first month after resection of the stomach. Variations in weight and food excitability were subsequently observed in this dog. Beginning the tenth month after the operation, food excitability declined sharply; the dog often refused to eat and after 11½ months was killed in an extremely emaciated condition, having lost 50% of his original weight.

As the data presented show, the changes in the dynamics of glucose and water absorption caused by resection of the stomach were expressed to different degrees in the different experimental dogs.

Therefore, the experiments conducted on isolated intestinal loops showed that stomach resection caused the amount of glucose absorption to decrease during the first month after the operation and that this index then increased and fluctuated within normal limits (with observations lasting from 3 months to 1½ years). Water absorption was considerably increased by the operation and remained so for a long time.

When the character of the glycemic curve after the per os administration of glucose is compared with glucose absorption in an isolated intestinal section (dog Laima), it is evident that the glycemic curve shows more pronounced changes after resection of the stomach; this seems to be connected with the fact that, as we have already mentioned, the glycemic curve reflects more than the glucose-absorbing function of the intestine.

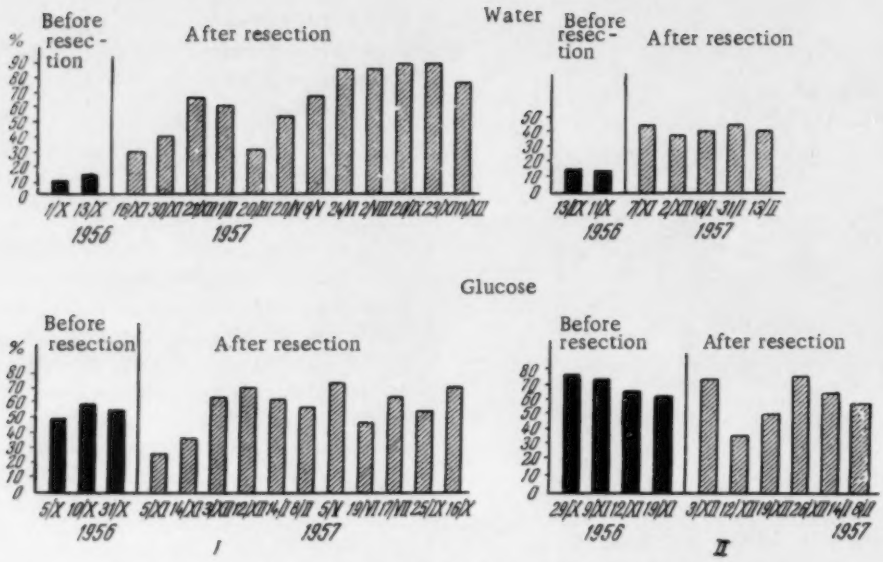

Fig. 3. Glucose and water absorption in the dogs Laima (I) and Trezor (II). Ordinate axis represents the amount of glucose and water absorption in percent of the quantity administered; the abscissa axis represents the days of the experiments (date).

At the same time, our experimental data showed the absorption of glucose and water, the blood sugar level and the character of the glycemic curve depend on the general condition of the dog after the resection of the stomach.

SUMMARY

The author describes the effect of partial resection of the stomach on the absorptive ability of the small intestine. Experiments were performed on dogs by 2 methods. The dynamics of glucose absorption were studied in normal conditions and at various time periods after resection of the stomach. Experiments were conducted by the administration of glucose into the digestive tract and subsequent study of the glycemic curve. The absorption function of the "fasting" small intestine was examined in 4 dogs with the isolated section of the superior portion of the small intestine by Thiry's and Thiry-Vella's methods. 0.5-1% glucose and water were given as "test meals". Experiments conducted by the first method demonstrated that after stomach resection, the blood sugar level (before meals) and the character of glycemic curve show considerable changes, but the changes are different in these two dogs. Experiments carried out on isolated intestinal sections show that as a result of stomach resection, the glucose absorption decreases during the first month after the operation, then rises and stays within the normal limits. The dogs were observed for from 3 months to 1½ years. Absorption of water is greatly increased after resection and remains in this condition for a long time. The absorption of glucose and water, as well as the blood sugar level and the character of glycemic curve, depends on the general condition of the animal after stomach resection.

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AN EXPERIMENTAL METHOD FOR ELECTROGRAPHICALLY DETERMINING THE MOTILITY OF THE DUODENUM DURING DIGESTION

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The secretory, excretory and motor functions of the gastrointestinal tract have been extensively studied in the laboratories of I. P. Razenkov. M. A. Sobakin used the electrographic method in his detailed study [2] of the motility of the small intestine wall in chronic experiments. We used the electroenterographic method simultaneously with the kymographic method to examine the motility of the duodenum during both physiologic hunger and digestion.

The problem concerning the parallel comparison of the electric and mechanical activity of the small intestine has been very inadequately treated in the literature. What works there are on this subject have been for the most part conducted under conditions of acute experiments or on isolated preparations of the small intestine (E. R. Mogilevskii, 1944; Berkson, 1933, Bozler, 1939; W. C. Alvarez and Mahoney, 1922; C. B. Puestow, 1932-1933; B. I. Hasama, 1934, et al.). The material obtained by these authors indicates that the rhythms of the electric and mechanical activity of the intestinal wall are coincidental. The first attempt in world literature to study the electric phenomena in the small intestine under conditions of the chronic experiment was that of A. A. Titaev (1943). In his investigations, special attention was given to potential changes connected with the secretory activity of the small intestine wall.

In the absence of chyme, good contact was assured by the method we used for leading off the biological currents from the intestinal mucosa. Electrographic determination of small intestine motility is considerably more difficult in the presence of chyme, because foreign particles of chyme can fall between the electrode and the intestinal wall during the pendular and peristaltic movements, altering the degree of contact and the exactitude of the recording.

For our study of duodenal motility, particularly during digestion, we used the method M. A. Sobakin developed [2] of leading off biological currents from an electrode implanted in the wall of the gastrointestinal tract. Recording was done with a unipolar lead. The biological currents were led off to an ink-writing EGS-1[3] apparatus. The ink recording, in contrast to a photogram, made it possible to observe the rhythmic activity occurring during digestion as it occurred. Under conditions of physiologic hunger, we synchronously recorded on the same tape the electric waves and the motility of the intestine as registered by a balloon-kymograph.

The tape was adjusted to two speeds in order to obtain a more extensive recording.

As the indifferent electrode, we used a bath with a warm physiological solution, with the posterior extremities of the animal submerged in it. The active electrode was a plastmass (methacrylate) fistula placed on the body of the stomach. From this, a multiple-strand wire insulated in vinyl chloride was passed through a large gasket. A platinum loop was welded to the end of this wire. This loop was introduced through a small incision in the seromuscular layer into the duodenal submucosa 4-5 cm below the tip of the pancreas. In order to keep the electrode firmly in the intestinal wall during peristalsis, a gasket was fastened to the implantation

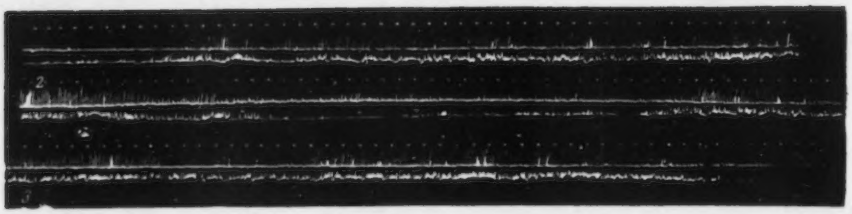

Fig. 1. Synchronous recordings of duodenal motility under conditions of physiologic hunger by electrography (top curve) and by kymography (bottom curve); time is shown in minutes by the dots; the calibration at the end of the recording is 10 mv. Experiment made November 21, 1957 on the dog Laima.

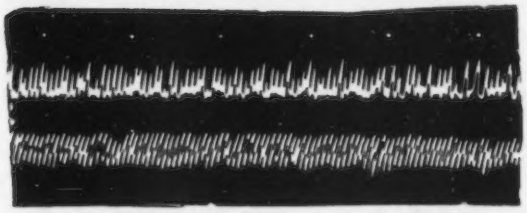

Fig. 2. Synchronous recordings of duodenal motility on high-speed tape under conditions of physiologic hunger. Curves (from top to bottom): time in one minute marks; electroenterogram, electromyogram.

place of the platinum loop.

To avoid the penetration of the platinum loop into the intestinal lumen, a plastmass deflector was attached above the platinum loop on top of the serous membrane of the intestine. The deflector and the platinum loop were fastened to the intestinal wall with ligatures of thin silk.

EXPERIMENTAL RESULTS

Electrodes implanted in this way made it possible to make prolonged and continuous observations in chronic experiments of intestinal motility both during digestion and on an empty stomach. Figure 1 shows a sample of the synchronous electrographic and kymographic recordings of duodenal motility on an empty stomach. As the Figure shows, the electric recording coincided with the mechanical in both the alternation of the "work" and "rest" period and the force and rhythm of the contractions. The "work" period of the duodenum lasted an average of 30-40 minutes. The rhythm of the peristaltic waves was 5 waves per minute, of the pendular movements, 16-18 waves per minute. When the periods of relative "rest" were compared, it was found that the mechanographic method of recording did not give a clear picture of the motor process. Evidently, this method does not reflect all the processes which take place in the intestinal wall at the various periods and with the various forms of its activity. Our electrographic data and the experimental material obtained by E. S. Mogilevskii in acute experiments give reason to propose that the rhythmic processes continue in the intestinal wall as fluctuations of the bioelectric potentials throughout the "rest" period, although no clear contractions are observed during this time.

These rhythms appeared more clearly on the higher-speed tapes (Fig. 2). Figure 2 gives a good illustration of how the rhythm of the electric waves and of the mechanical contractions, 16 waves per minute, i. e. corresponding to the pendular movements, is coincidental. During the whole "work" period, however, no such true conformity was observed, because the electrographic recording method is more sensitive than the mechanographic. For example, we included an electroenterogram made during digestion (Fig. 3). The food stimulus (300 g of bread) was given during a "rest" period, 10 minutes after the termination of a "work" period. After the ingestion of the food stimulus, the amplitudes of the electric waves, and therefore the force of the duodenal contractions, showed a definite variability. The alternation of the groups of higher-amplitude electric waves representing the peristaltic and pendular movements was similarly variable in time. However, as a whole, the electric activity of the duodenal wall is greater during digestion than during the periods of relative "rest" and less than during the "work" periods in conditions of physiologic hunger.

SUMMARY

The author developed a method of leading off the biocurrents from the implanted electrodes. The latter provide the possibility of carrying out prolonged and continuous electrographic examinations of the

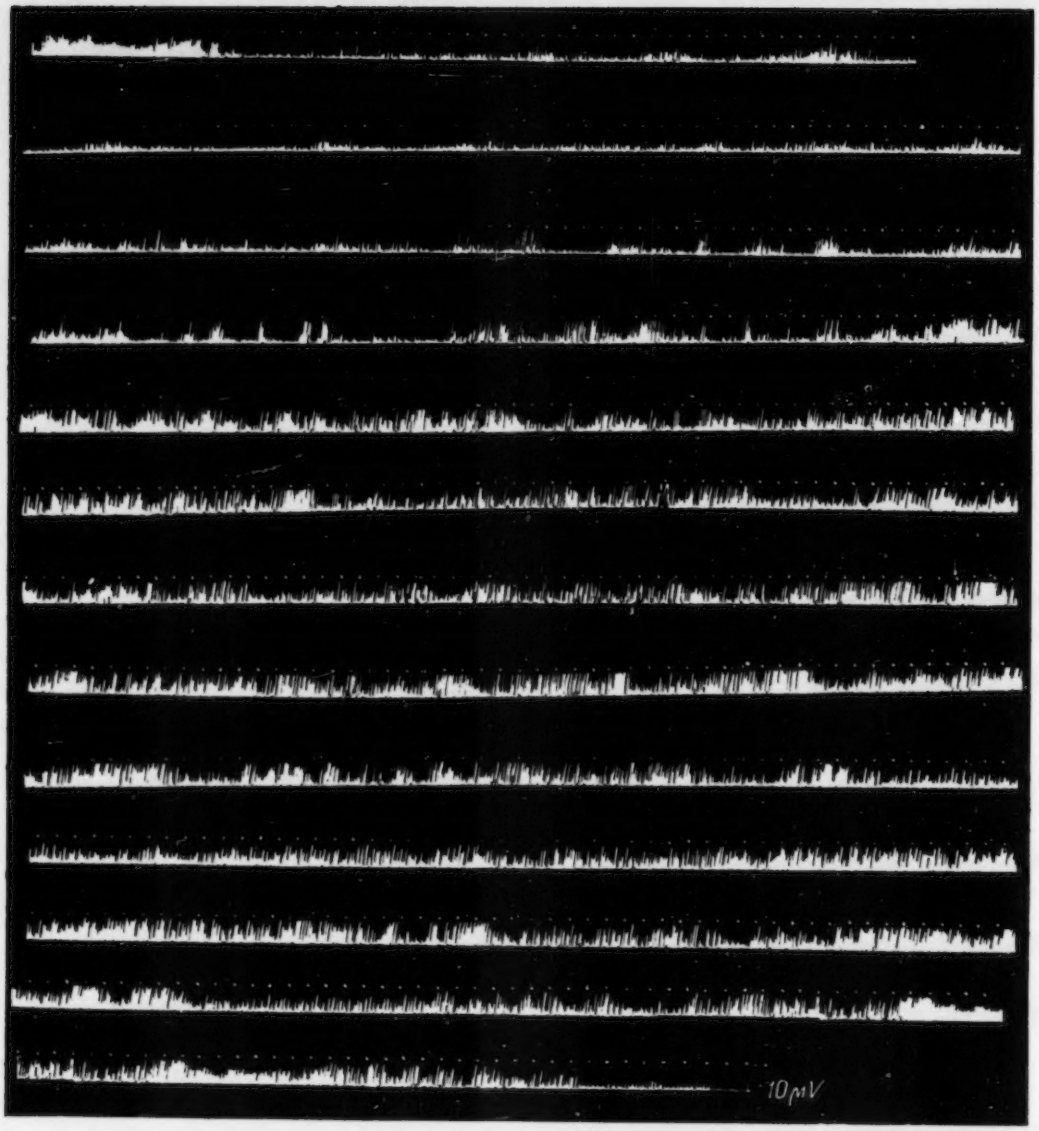

Fig. 3. Electroenterogram of duodenal motility during digestion. Food stimulus (300 g of bread) given during a "rest" period 10 minutes after the termination of the preceding "work" period. Time shown in one-minute marks above the recording. Calibration 10 mv. Experiment made February 25, 1958 on the dog Atlas.

intestinal motor function before meals and during digestion in conditions of chronic experiments. There is an electric mechanographic registration of the periods of "work" alternated by the periods of "rest", as well as of the rhythm and power of contractions. The period of duodenal "work" is equal to 30-40 minutes, on the average. The rhythm of the peristaltic waves is equal to 5 oscillations per minute, while the rhythm of the pendular motions is equal to 16-18 per minute. The period of relative rest is equal to 50-80 minutes, on the average.

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THE EFFECT OF RADIAL ACCELERATIONS ON SALIVARY AND GASTRIC GLAND SECRETION AND ON THE PERIODIC CONTRACTIONS OF THE STOMACH

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The problem of the effect of radial accelerations on the human organism is more than 150 years old. It was first created by the use of radial accelerations in the treatment of various diseases [21], becoming a problem of great importance with the development of high-speed aviation; during both these periods, most of the research on this problem concentrated on the changes which occur in the circulatory and respiratory systems [9, 10, 12, 13, 18, 22, 23], although some research has also been done on the condition of the central nervous system [2, 14, 20]. There has, however, been hardly any investigation of the changes which occur under these conditions in the other internal organs, specifically the digestive system. There is one work [24] which indicates that certain changes in the digestive system expressed as pains in the epigastric region, loss of appetite and fatigue have appeared in many flyers who have been working for a specific period with high-speed airplanes. When these pilots were transferred to slower machines, these symptoms disappeared. No special experimental investigations have been conducted in this direction. Meanwhile, the development of jet aviation and of the problem of interplanetary communication have put the resolution of the subject problems on the agenda.

The purpose of this investigation was to study the effect of radial accelerations on secretion by the salivary and gastric glands and on the periodic contractions of the stomach.

EXPERIMENTAL METHODS

The work was performed on 6 dogs never before subjected to the action of radial accelerations. A total of 310 experiments were conducted.

We studied the effect of radial accelerations on reflex salivation induced by food (10 g of bread, 5 g of discuit) and inhibitory (10 ml of a 0.25% solution of hydrochloric acid) stimuli with three dogs (Mishka, Lis and Nochka), in which the salivary gland ducts were exposed according to D. L. Glinskii's method [3]. The unconditioned stimulus was used 4 times during the experiment at 15 minute intervals. It was used for 15 seconds each time.

Salivation was recorded for 15 minutes from the time the unconditioned stimulus was used.

Besides determining the amount of saliva secreted, we also determined the activity of the enzyme ptyalin according to the Wohlgemuth method [25] and the dense residue. The number of milliliters of a 1% solution of starch split into dextrins during a 30-minute period by 1 ml of saliva at a temperature of 38° was used as the conditional measure of amylase activity.

We used a special irrigation system (Fig. 1) to study the reflex salivation during the action of radial accelerations. When the electromagnet D was connected to the current from the accumulator A, the acid dropped from the glass vessel B into the waste pipe. It was therefore possible to regulate acid irrigation during the action of the radial accelerations.

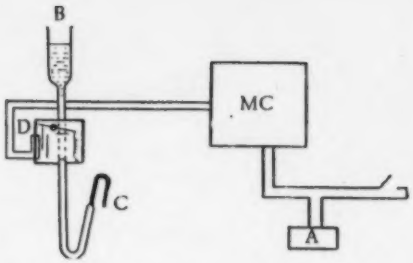

Fig. 1. Plan of irrigation system.
A) accumulator; B) glass vessel with acid; C) waste pipe; MC) mercury centrifuge receptacle.

The effect of radial accelerations on the secretion and periodic contractions of the stomach was studied on the other three dogs, two of which had Basov's gastric fistulas (Trusikha, Pegaia) and one, a Heidenhain's isolated stomach pouch (Mal'chik). The periodic contractions were recorded in the dogs with Basov's gastric fistulas by means of the usual balloon method, secretion being simultaneously observed. The experiment lasted 4 hours. Secretion was induced in the Heidenhain's isolated stomach pouch by the administration of 600 ml of milk. In this case, secretion was observed for 6 hours, starting from the time the milk was administered.

Radial accelerations were created by revolving the animals on a centrifuge 3.6 m in radius. In the experiments, we used radial accelerations* of 3-9 g in a craniocaudal direction, lasting 20 or 60 seconds and accelerations of 3-5 g in a caudocranial direction lasting 20 seconds.

A background of the glandular secretion and periodic stomach contractions was established for 1-1½ months before the experiments with the effect of the accelerations on the animals. The animals were fed a mixed diet during the experimental period and were starved for 18 hours before each experiment.

TABLE 1

Record of Experiment No. 87, Made July 27, 1957 on Dog Lis

Conduction time of experiment	Stimulus	Amount of saliva (in ml)	Remarks
16^{00}-16^{02}	Hydrochloric acid	1.3	Acid irrigation done before rotation was started
16^{02}-16^{15}	No stimulus	0.2	
16^{15}-16^{17}	Hydrochloric acid and influence of craniocaudally directed accelerations of 3 g, lasting 60 seconds	0.6	Acid irrigation begun the 15th second of the rotation period
16^{17}-16^{30}	No stimulus	0.1	
16^{30}-16^{32}	Hydrochloric acid	1.2	Acid irrigation done at rest
16^{32}-16^{45}	No stimulus	0.1	
16^{45}-16^{47}	Hydrochloric acid and influence of craniocaudally directed accelerations of 3 g, lasting 60 seconds	0.2	Acid irrigation begun the 15th second of the rotation period

EXPERIMENTAL RESULTS

The experiments studying parotid gland secretion showed a considerable inhibition of reflex salivation during the action of the craniocaudally directed radial accelerations. We included the record of an experiment performed on the dog Lis as a typical example (Table 1).

* In aviation medicine, the rate of radial accelerations is usually expressed in terms of acceleration of gravity (g), i. e. 9.81 meters per sec.^{2}, and the direction of their action is usually taken as the direction of the inertia forces. Thus when the direction is described as craniocaudal, it means that the action of the inertia forces is directed from the head towards the pelvis. This terminology is employed in this paper.

TABLE 2

Effect of Craniocaudally Directed Radial Accelerations on Secretion of Dogs' Parotid Glands

Experimental conditions / Indices of secretion	Dog Lis			Dog Nochka		
	30/X 1956	31/X 1956	1/XI 1956	20/XII 1956	22/XII 1956	24/XII 1956
	background	after* 7g accel 20 sec.		background	after* 7g accel / min.	
	bread	bread	bread	HCl	HCl	HCl
Amount of saliva (in ml) secreted with the 1st use of the unconditioned stimulus	0,4	0,2	0,4	0,6	0,1	0,6
Amount of saliva (in ml) secreted with the 2nd use of the unconditioned stimulus	0,4	0,2	0,4	0,7	0,6	0,9
Amount of saliva (in ml) secreted with the 3rd use of the unconditioned stimulus	0,4	0,4	0,5	1,0	0,5	0,8
Amount of saliva (in ml) secreted with the 4th use of the unconditioned stimulus	0,6	0,2	0,4	0,6	1,0	0,7
Amount of saliva (in ml - total for experiment)	1,8	1,0	1,7	2,9	2,2	3,0
Dense residue (in %)	0,52	0,50	0,65	0,70	0,86	1,18
Amylase (in conditional units)	0	2	0	0	4	2

* Investigation of saliva secretion began 3 minutes after the termination of rotation.

As the record shows, salivation to hydrochloric acid sharply decreased during the rotations. When the rotations were repeated, the inhibition of secretion caused was even more pronounced (about 0.2 ml of saliva, instead of the 0.6 ml secreted during the first rotations). Simiar results were observed with the other dogs.

There was a brief aftereffect (inhibition of reflex salivation) after the action of craniocaudally directed radial accelerations of 5-9 g for 20-60 seconds. Amylase appeared in the dogs' saliva with the first use of the accelerations (Table 2).

As Table 2 shows, in the October 31, 1956 experiment on the dog Lis, 0.2 ml of saliva was secreted with the first and second use of the unconditioned stimulus (3.18 minutes after rotation) after the 20-second influence

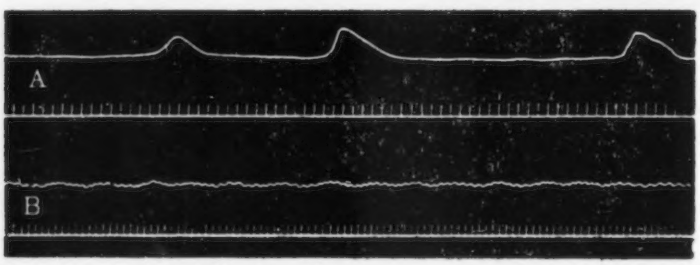

Fig. 2. Periodic contractions of the stomach in the dog Pegaia before (A) and after (B) the action of craniocaudally directed radial accelerations of 7 g for 20 seconds. Lower curves – indication of time in 3 second marks.

of 7 g radial accelerations, instead of the usual 0.4 ml observed in the experiments made before rotation. Amylase was found in the saliva. Th. dense residue was unchanged.

Analogous data were obtained in the experiments on the dog Nochka.

After the action of caudocranially directed accelerations, salivary inhibition was induced even by 20-second accelerations of 3 g.

Accelerations of 5 g caused a longer aftereffect (lasting about 24 hours). The duration of the aftereffect depended on the animal's type of nervous activity. The aftereffect disappeared sooner in the dogs with active nervous processes (Mishka) than in the dogs with passive nervous processes (Lis, Nochka).

Therefore, the action of radial accelerations causes acute inhibition of saliva secretion during rotation, which disappears soon after the termination of rotation. Only after the action of caudocranially directed accelerations of 5 g for 20 seconds was salivation observed to be still inhibited on the following day.

One should note that the quantitative changes in secretion caused by the first rotations were attended by the appearance of amylase in the saliva. This could indicate the presence of changes in the trophic processes. In I. P. Razenkov's laboratory, this phenomenon was observed in dogs under the influence of oxygen starvation, as when the animals were fed a primarily carbohydrate diet. It has been shown that the appearance of amylase in the saliva is connected with changes in the sympathetic innervation of the salivary glands as well as with changes

TABLE 3

Effect of Caudocranially Directed Radial Accelerations on the Secretion and Periodic Contractions of the Dog Trusikha's Stomach

Experimental conditions / Time		Background			Action of accelerations		
		date of investigation					
		14/XII 1956			19/XII 1956		
		stomach contractions	secretion from fistula (in ml)	reaction to litmus	stomach contractions	secretion from fistula (in ml)	reaction to litmus
1st hour	0—15 minutes	—	1.0	Alkali	+	0	—
	15—30 »	+	1.0	»		5 g accel/20 sec.	
	30—45 »	—	1.0	»	—	0	—
	45—60 »	—	1.0	»	—	2.5	Acid
2nd hour	0—15 minutes	—	1.0	Alkali	—	5.0	Acid
	15—30 »	—	1.0	»	—	1.5	»
	30—45 »	—	2.0	»	—	1.5	»
	45—60 »	+	0.5	»	—	1.5	»
3rd hour	0—15 minutes	—	0.5	Alkali	—	5.0	Acid
	15—30 »	—	0.5	»	—	8.0	»
	30—45 »	—	1.0	»	—	3.5	»
	45—60 »	+	1.0	»	—	3.5	»
4th hour	0—15 minutes	—	1.0	Alkali	—	5.0	Acid
	15—30 »	—	0.8	»	—	5.0	»
	30—45 »	—	0.7	»	—	5.0	»
	45—60 »	—	1.5	»	—	5.0	»

Symbols: + period of stomach contractions present; — presence of rest periods.

in the functional condition of the higher divisions of the central nervous system [5, 11, 15, 16]. One can propose that when these changes occur due to the influence of radial accelerations, they are also connected with changes in the functional conditions of the central nervous system, which are caused by hypoxia and by increased centripetal pull from various organs and tissues which are deformed during the action of the accelerations.

The second part of the work was dedicated to the study of the effect of radial accelerations on the secretion and periodic contractions of the stomach. The experiments performed for this purpose showed that the changes observed took the same general course as those observed for the salivary glands. Thus, the complete disappearance of 1-2 periods of stomach contractions was observed after the 20-second action of craniocaudally directed radial accelerations of 5-7 g (Fig. 2).

Figure 2, B, shows a typical curve recording gastric motility in the dog Pegaia after the action of 7 g acceleration.

The repeated action of accelerations of this force usually caused only a decrease in the amplitude of the stomach contractions in the first period after the rotation.

Analogous changes were observed with the action of caudocranially directed accelerations of 3 g. In these conditions, there was no change in the secretion of the empty stomach. However, 20-second, caudocranially directed accelerations of 5 g and 60-second, craniocaudally directed accelerations of 7-9 g caused the spontaneous secretion of gastric juice (Table 3).

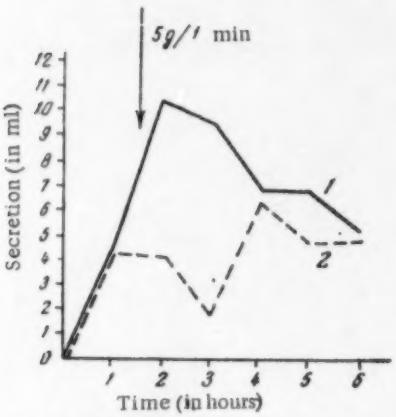

Fig. 3. Effect of 1-minute, craniocaudally directed radial accelerations of 5 g on secretion in a Heidenhain's isolated stomach pouch in an experiment on the dog Mal'chik.
1) control, 2) experiment.

As Table 3 shows, after the action of caudocranially directed accelerations of 5 g, the periodic contractions of the stomach disappeared, while profuse secretion of acid gastric juice was observed after 15 minutes. Analogous results were obtained with the 60-second action of craniocaudally directed accelerations of 7 g. Therefore, although craniocaudally directed accelerations cause hemodynamic changes opposite to those caused by caudocranially directed accelerations [19, 23], the two types of accelerations cause similar changes in the secretory and periodic activity of the stomach. The same was observed to be true of saliva secretion. This fact shows that the changes observed in the secretory and periodic activity of the stomach must be primarily due to changes in the regulatory mechanisms. The main secretory nerve of the stomach is known to be the vagus nerve [7, 8, 17]. Therefore, the development of spontaneous secretion observed with the action of the accelerations could be due primarily to stimulation of the vagus nerves. We studied the secretion in a Heidenhain's isolated stomach pouch in order to verify this proposal. We did not observe the appearance of spontaneous secretion in these experiments. Only a prolonged inhibition of juice secretion (lasting $1\frac{1}{2}$-$2\frac{1}{2}$ hours) was observed (Fig. 3).

Therefore, these results confirm the above proposal concerning the role of the vagus nerves and also indicate the presence of changes in the sympathetic nervous system (prolonged inhibition of secretion in the Heidenhain's stomach pouch); the latter also concurs with the data of other authors [1, 4, 6].

SUMMARY

The author studied the effect of radial accelerations on the secretion of parotid glands, gastric glands and on the periodic stomach contractions. Radial accelerations in the craniocaudal and caudocranial direction caused inhibition of reflex salivary secretion and of the periodic stomach contractions. A spontaneous secretion of the gastric juice was noted in high accelerations in dogs with a Basov stomach fistula. Disturbance

of the parasympathetic innervation in a dog with an isolated Heidenhain's stomach pouch resulted in the absence of spontaneous gastric secretion. However, a prolonged inhibition of gastric secretion was noted in these conditions. It is assumed that the changes described above are connected with changes in the nervous system, particularly in its parasympathetic and sympathetic portions.

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TYPE OF HIGHER NERVOUS ACTIVITY CLASSIFIED ACCORDING TO FOOD AND ACID-DEFENSE CONDITIONED REFLEXES

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The question concerning the comparative typological characteristics of higher nervous activity according to the results obtained from studying the dynamics of conditioned reflexes based on different unconditioned stimuli has interested certain authors for a long time. A. G. Ivanov-Smolenskii [2] and his co-workers [3, 6] established that the typological properties can vary as to character in the same child, depending on the type of reinforcement used. For example, the use of defense reflexes could classify the child as the passive type, while according to food reflexes, the same child could be found to be the active type.

A. P. Chestnokova [7], M. S. Alekseeva [1] and A. M. Klochkov [4], who compared the typological properties of dogs according to food and acid-defense conditioned reflexes, found that the differentiation of conditioned stimuli and the alteration of their signal meaning occurred more slowly and with great fluctuations in one animal than when food reinforcement was used with the same animal. The fact that unitypical conditioned reflex processes can occur differently with different unconditioned reinforcements, i. e. do not coincide as to quantitative parameter, is somehow not very surprising. Our purpose was to demonstrate the character of these differences in order to facilitate the determination of an animal's typological properties.

This article presents the results obtained from experiments determining the type of higher nervous activity possessed to two dogs (Belyi and Chernyi), using two methods simultaneously, i. e. the food-secretomotor and acid-defense methods.

EXPERIMENTAL METHODS

The experiments were performed twice a day, by the food method in the morning and by the acid-defense method in the evening. This experimental plan enabled us to keep the experimental results from being affected by possible changes in the general condition of the animals, as would have occurred had type determination been done first by one method and then by the other (as was done by the authors cited above).

In our experiments, the food conditioned stimuli differed from the acid-defense, but the differentiations were selected so that the differentiation conditions would be approximately equal in both cases. Type determination followed a program similar to the abbreviated standard of M. S. Kolesnikov and V. A. Troshikhin [5].

Food Method. Positive food-secretomotor conditioned reflexes developed in Belyi and Chernyi at the 14th and 15th combinations, respectively. The dependence of the effect on the strength of the conditioned stimulus was well expressed.

A differentiating inhibition was formed especially quickly in Belyi. The motor reaction (running up) to the food dish was inhibited by the 12th use of the negative stimulus, and the secretory component, by the 18th use. After this, the differentiation was, as a rule, absolute (Fig. 1, a). The differentiation was slower to develop in Chernyi, and then only relatively absolute (Fig. 1, a). The amount of conditioned secretion to the inhibitory

TABLE 1

Change Effected by Caffeine in Secretory Conditioned Reflexes of Dogs

Experimental dogs	Experiment the evening before the use of caffeine	Caffeine, in a dose of 0.3 g	Experiment the evening before the use of caffeine	Caffeine, in a dose of 0.6 g
Belyi	145 / 0	153 / 0	156 / 0	189 / 0
Chernyi	171 / 26%	176 / 30%	166 / 34%	208 / 76%

Note: The figure above the line is the sum of the positive conditioned reflexes in scale divisions; the figure below the line is the differentiation in percent of the positive reflex.

stimulus was, on the average, 20-30% of the amount of secretion to the positive stimulus. In most cases, the motor reaction was inhibited, or was expressed by uncompleted running approaches (with the animal returning to its original place before reaching the food dish).

We used caffeine in doses of 0.3 and 0.6 g in order to test the force of the stimulating and inhibitory processes.

EXPERIMENTAL RESULTS

The first dose (0.3 g) of caffeine did not noticeably affect the animals' conditioned reflex activity. The 0.6 g dose of caffeine considerably enhanced the positive secretory conditioned reflexes in both animals. The caffeine caused a considerable disinhibition of the differentiation in Chernyi, but did not affect the differentiation in Belyi (Table 1). No disturbances in conditioned reflex activity were observed in the experiments performed subsequently.

The strength of the inhibitory process was tested by prolonging the action of the differentiation stimulus to 3 minutes; this did not cause any significant disinhibition of the differentiation in Belyi. In this dog, the dynamics of salivation were, in scale divisions (every 30 seconds), as follows: 5-3-3-3-0-0. In Chernyi, this experiment caused extensive disinhibition of both the secretory and motor components of the food reflex. Salivation every 30 seconds was 16 – 15 – 9 – 9 – 12 – 0 scale divisions. After these experiments, conditioned reflex

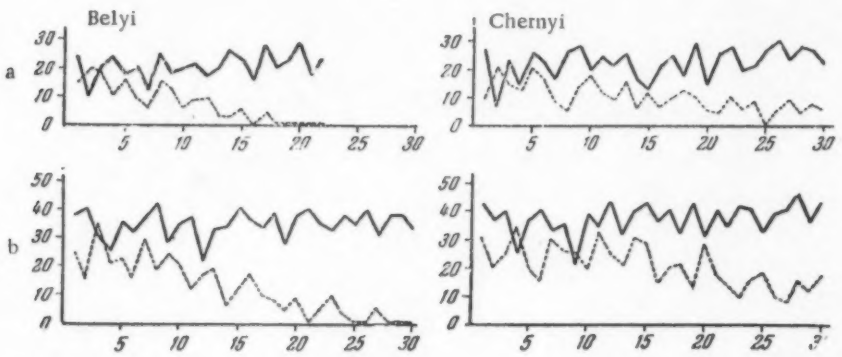

Fig. 1. Development of differentiating inhibition.
a) With use of food method; b) with use of acid-defense method.
Symbols: The ordinate axis represents the conditioned secretion in scale divisions; the abscissa axis represents the ordinal number of the use of the conditioned stimuli. Solid line – positive reflex; dotted line – inhibitory reflex.

TABLE 2

Change Effected by Caffeine in Defense Conditioned Reflexes of Dogs

Experimental dogs	Experiment the evening before the use of caffeine	Caffeine, in a dose of 0.3 g	Experiment the evening before the use of caffeine	Caffeine, in a dose of 0.6 g
Belyi	201 0	211 0	205 0	234 0
Chernyi	229 36%	240 50%	233 43%	257 91%

Note: Figures mean the same as in Table 1.

activity again proceeded normally.

The lability of the nervous processes was determined by altering the signal meaning of the conditioned stimuli. Belyi found this easy to cope with, which showed that the lability of the nervous processes was good (Fig. 2, a). Chernyi took longer to comprehend the alteration; he comprehended the alteration of the inhibitory stimulus' meaning more quickly than that of the positive (Fig. 2, a).

On the basis of the experiments performed, Belyi could be described as an animal of the strong, balanced and mobile type. Chernyi could also be classified as the strong type, but with nervous processes which were excitable and of average lability. A considerable predominance of the stimulation process over the inhibitory was characteristic of this animal, as was clearly shown by all the indices of conditioned reflex activity.

Acid-Defense Method. Positive conditioned reflexes were developed in Belyi and Chernyi by the 12th and 10th combinations respectively. The dependence of the conditioned reflex effect on the strength of the stimulus was as well expressed as with the food reinforcement. The acid-defense conditioned reflexes exceeded the food reflexes in value by an average of 25% in Belyi, and by an average of 35% in Chernyi.

Differentiating inhibition was slower to develop and less profound under conditions of acid reinforcement than with the food reinforcement. As with the food method, the differentiation was absolute with the defense reflexes in Belyi, but was slower to develop and somewhat less stable (Fig. 1, b). In Chernyi, the differentiation was relative in both cases, but even more so with the acid reinforcement; the differentiation reflex was 35-40% of the conditioned reflex (Fig. 1, b).

In a dose of 0.3 g, caffeine slightly enhanced the conditioned reflexes in the experimental animals; only in Chernyi was the differentiation slightly disinhibited.

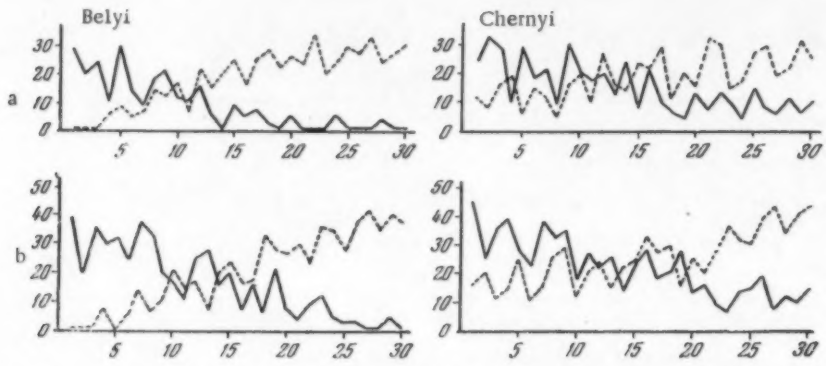

Fig. 2. Alteration of signal meanings of the conditioned stimuli.
a) With food method; b) with acid-defense method; dotted line – positive reflex; solid line – inhibitory. Symbols are the same as in Figure 1.

In a dose of 0.6 g, caffeine further enhanced the positive conditioned reflexes. The differentiation was slightly disinhibited in Belyi (Table 2). There was a strong disinhibition of the differentiation and an increase in general motor activity in Chernyi.

The experiments did not affect in any way the conditioned reflex activity of the animals in subsequent experiments.

In the experiment prolonging the action of the differentiation stimulus to 3 minutes, a strong and stable inhibition process was observed in Belyi, as in the analogous experiment made under conditions of the food method.

Salivation every 30 seconds in Belyi was 7-3-8-0-3-4-0, in scale divisions. In Chernyi, this experiment had about the same results as that with the food reinforcement, i. e. strong disinhibition of the differentiation was observed. The course of salivation every 30 seconds in scale divisions was as follows: 25 – 30 – 16 – 8 – 0 – 20 – 5. On the day after this experiment, the differentiation was observed to have somewhat deteriorated in Chernyi.

Alteration of the signal meanings of the conditioned stimuli was comprehended by both dogs somewhat more slowly in the experiments with the acid reinforcement than in those with the food reinforcement. However, in this case also, Belyi manifested very labile nervous processes and became completely adjusted to the change (Fig. 2, b). The lability of the nervous processes was found to be low in Chernyi (Fig. 2, b), as in the experiments with food reinforcement.

A comparison of the experimental data obtained under the two kinds of experimental conditions showed that in both cases, both dogs were shown to belong to the strong type of nervous activity. The main differences between the two dogs in respect to balance and lability of the nervous processes were equally apparent under both sets of conditions. We estimate the typological properties of an animal, of course, according to the pertinent quantitative indices of its conditioned reflex activity, which indices are established during the accomplishment of experimental tasks. However, as we have seen, the quantitative indices characterizing the resolution of the same experimental problem can vary in the same animal according to the type of unconditioned reinforcement used. By no means does it follow from this that it is a question of varying typological properties in the same animal.

A single type can certainly manifest these differences under different conditions which evidently require different exertion of the cortical nervous functions.

Thus, for example, an animal can possess an inhibitory process strong enough for the development of a stable differentiating inhibition under conditions of the food method, yet not strong enough to form as stable a differentiation under conditions of the acid-defense method. The same can be said of the lability of the nervous processes. The question concerning the possible reasons for such interrelations requires special discussion.

Therefore, the results of the experiments conducted show that experimental data regarding type of higher nervous activity and obtained by both the food and acid-defense methods reflect the typological features of the same animal synonomously.

SUMMARY

A parallel determination of the type of the highest nervous activity was carried out in two dogs by two different methods (by food secretory motor and by acid-defense). According to the data of the food method, one dog appeared to be of a strong, well balanced and labile type, while the other was a strong, excitable type with an average lability of the nerve processes. Analogous typological characteristics of these animals were also established by the acid-defensive method.

Thus, the results of these experiments demonstrated that data obtained by the food and acid-defense methods, reflect the typological characteristics of animals in the same way.

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THE EFFECT OF FOOD STIMULI UPON THE QUANTITY OF LEUKOCYTES IN HUMANS

COMMUNICATION II. THE DEPENDENCE OF CONDITIONED AND COMPLEX REFLEX FOOD LEUKOCYTOSIS ON FOOD EXCITABILITY AND ON THE INITIAL LEVEL OF LEUKOCYTOSIS*

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Our studies of the effect of various kinds of food on conditioned and complex reflex food leukocytosis have shown that both types of leukocytosis are more pronounced with the ingestion of sausage, salted herring, and vegetable soup (with meat or fish) than with the ingestion of white bread or pastry, especially the latter. At the same time, we found that this correlation could not always be observed. In some cases, conditioned and complex reflex food leukocytosis did not appear with the use of the first three food stimuli, but were clearly evident with the use of the last two.

This article analyzes some of the reasons for this discrepancy between the kinds of food stimuli and the degree to which the experimental forms of leukocytosis are expressed.

Analyzing the factors influencing the two types of leukocytosis with the use of different kinds of food, we turned our attention to the significance of food excitability in the experimental subjects. It has, for example, been shown that acute inhibition of food excitability is present, in some cases, both forms of leukocytosis are absent with the use of sausage, herring,and vegetable soup, as well as with the use of bread or pastry. When food excitability is comparatively high, however, even white bread or pastry can often cause marked conditioned and complex reflex food leukocytosis.

EXPERIMENTAL RESULTS

The table gives the results obtained in our study of these two types of leukocytosis under two sets of conditions: under conditions of heightened food excitability on the one hand, and under conditions of average or diminished food excitability on the other.

The effect of the status of food excitability on the experimental types of leukocytosis could be judged by comparing the data obtained from the subjects with heightened food excitability with the data from subjects with diminished food excitability. In the first case, both types of leukocytosis were usually present with the use of the most different kinds of food, while in the second case, both types were often absent. Thus, for example, in N-na, a 53-year-old subject, food excitability was remarkably high, as the subject herself confirmed, saying she had always been noted for "my good appetite". We also made observations on I-n, 35 years old, who usually ate the food set before him without any particular appetite. In the case of N-na, conditioned food leukocytosis was found in 16 out of 21 experiments and complex reflex leukocytosis 18 times. In I-n, the first type of leukocytosis was observed in 10 out of 21 experiments, the second type, in 15.

This dependence of conditioned and complex reflex food leukocytosis on the condition of food excitability was consistently observed in repeated observations on the same experimental subjects. As an illustration of

* The terms "conditioned and complex reflex" as in original text – Publishers.

Effect of Food Excitability on Food Leukocytosis

Type of food	Condition of food excitability	Number of subjects	Food leukocytosis			
			conditioned		complex reflex	
			present	absent	present	absent
Sausage	Heightened	36	32	4	33	3
	Average or diminished	11	4	7	4	7
Herring	Heightened	29	24	5	22	7
	Average or diminished	9	1	8	6	3
Vegetable soup	Heightened	15	13	2	15	–
	Average or diminished	16	2	14	12	4
White bread	Heightened	13	11	2	11	2
	Average or diminished	35	6	29	10	25
Pastry	Heightened	26	9	17	17	9
	Average or diminished	6	1	5	3	3

this, we cite the data obtained from observations on Gr-va, 18 years old, who constantly complained of her lack of appetite. However, she appeared for 2 of the 10 investigations after taking a long afternoon walk and ate the food she was given with great pleasure (both times, tea and a piece of white bread). In both of these 2 cases, the white bread caused both conditioned and complex reflex food leukocytosis, while with the sausage and herring eaten in other experiments, the first type of leukocytosis was often absent, and the second only weakly expressed (especially with the sausage).

Another important factor to be considered in order to explain the nonconformity of the experimental types of leukocytosis with the kind of food eaten is the original level of leukocytosis. We found, for example, that if there was an increase in the total number of white blood corpuscles before the food was exhibited or eaten, either a slight increase or even inhibition of both types of leukocytosis was usually observed.

Figure 1 shows the percentage of investigations in which conditioned and complex reflex food leukocytosis appeared in relation to the original levels of leukocytosis. This graph shows that these two forms of leukocytosis appeared less often as the original level of white blood corpuscles increased. More specifically, conditioned food leukocytosis was observed in 84% of the investigations with an original level of 3000-5000 leukocytes per 1 mm^3, and complex reflex leukocytosis, in 94%. The first type of leukocytosis was observed in only 21% of the investigations in which the initial leukocyte level was 9000-11,000 per 1 mm^3, however, and the second type, in 44%.

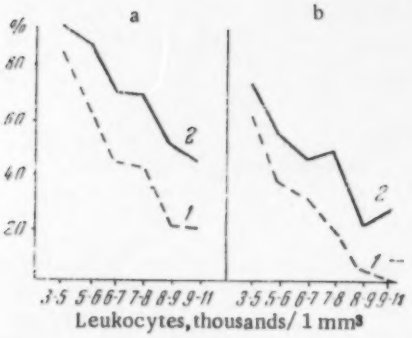

Fig. 1. Degree of increase in conditioned and complex reflex food leukocytosis. a) More than 1% of the original level of leukocytes; b) more than 25% of the original level of leukocytes; 1) complex reflex food leukocytosis; 2) conditioned food leukocytosis.

The importance of the original level of white blood corpuscles in determining the character of the conditioned and complex reflex food leukocytosis was also shown by repeated investigation of leukocytosis during the exhibition and intake of food. Leukocytosis was examined 2-3 and 4-5 minutes after eating began, in the same experimental subjects. These determinations showed that the greater the increase in leukocytosis shown by the first blood sample (as compared with the original level of leukocytes), the more leukocytosis in the second blood sample decreased (with both the exhibition and eating of food).

Figure 2 shows the percentage of investigations in which the total number of leukocytes in the second blood sample taken was observed to have decreased, depending

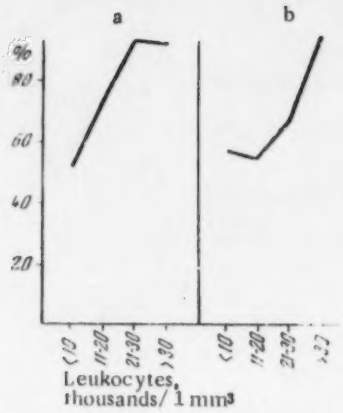

Fig. 2. Data from investigation of conditioned (a) and complex reflex (b) food leukocytosis.

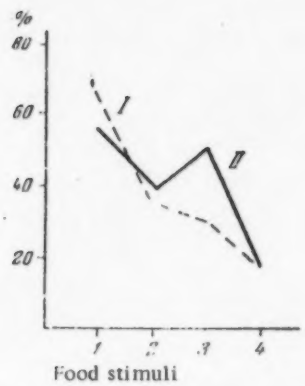

Fig. 3. Number of investigations in which conditioned (I) and complex reflex (II) food leukocytosis were inhibited by the influence of different food stimuli.

on the degree to which leukocytosis had been enhanced in the first sample taken – less than 10%, 11-20%, 21-30% and over 30% of the leukocytosis present before the exhibition of the food.

There was a substantial difference between the percentage of investigations with leukocytosis increased by less than 10% in the first blood sample in which leukocytosis decreased in the second sample and the corresponding percentage of investigations in which leukocytosis was more than 30% higher in the first sample than the original level. In the latter case, the percentage of the investigations showing a decrease, as the curves in Figure 2 show, was almost twice as large as in the former case (this was true for both types of food leukocytosis). The results of our observations on the influence of the original level of white blood corpuscles on food leukocytosis in people coincide with the data which have been obtained with animals [4 et al.]

According to K. M. Bykov [1], the activity of various internal organs is usually inhibited by the action of positive cortical impulses on these organs during their activity. There is every reason to consider the decrease which occurred in conditioned and complex reflex food leukocytosis when the original leukocyte level was high in the light of the proposition expressed by K. M. Bykov.

A series of works have demonstrated that the inhibition of a given organ's activity caused by different influences depends not only on the intensity of the organ's activity at the time, but also on the strength of the influential factors. To a certain extent, our research confirmed these observations. It was found, for example, that when the initial level of white blood corpuscles was comparatively high, the influence of such food stimuli as sausage, herring and vegetable soup caused a more rapid inhibition of conditioned and complex reflex food leukocytosis than did the intake of white bread alone, which was eaten by the subjects reluctantly. Figure 3 illustrates this by means of curves showing the percentage of investigations in which the two types of leukocytosis became inhibited with the subject's intake of sausage (1), herring (2), vegetable soup (3) and white bread (4). The original leukocytosis was over 8000 per 1 mm^3 in all of these cases.

The data we have presented clearly show that the type of food ingested must be taken into account, as well as the amount of leukocytosis, when estimating the reasons for decreased conditioned and complex reflex food leukocytosis under conditions of a relatively high initial level of leukocytes.

SUMMARY

This work presents data concerning the effect of different food excitability ("appetite") as well as of the initial level of leukocytosis on the conditioned and complex reflex food leukocytosis. In presence of increased food excitability, the conditioned and complex reflex food leukocytosis is much more pronounced than when this excitability is decreased. The degree of both types of leukocytosis also depends on such an important factor as the initial level of white blood cells. It was shown that with comparatively high initial values of leukocytosis, the absence of conditioned and complex reflex food leukocytosis is much more common than with a low level of white blood cells (even in the presence of high food excitability). Depression of these forms of leukocytosis in cases of increased initial level of white blood cells is especially pronounced with the action of

food stimuli such as sausage, salted herring and vegetable soup.

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CONCERNING THE BACKGROUND ELECTRIC ACTIVITY OF A RABBIT'S SPINAL CORD (A PUNCTURE METHOD OF LEADING OFF THE BIO-ELECTRIC POTENTIALS OF THE SPINAL CORD)

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The spinal cord is considered to be one of the most thoroughly studied sections of the central nervous system; nevertheless, our knowledge of the interconnection of the spinal nervous mechanisms with the other sections of the central nervous system is far from complete.

In Charles Sherrington's classic works, as in numerous subsequent electrophysiological investigations, experimental conditions were purposely created to provide isolation of the spinal cord, or even of a spinal reflex arc. The use of these methods made it possible to analyze many phenomena and contributed many valuable facts to our knowledge. However, these facts must be verified to some extent before the activity of the central nervous system in an intact organism can be accurately evaluated.

There have recently appeared several works studying the influence of the higher sections of the central nervous system on the processes in the spinal reflex arcs induced by specific stimulation [6, 7, 11, 15, 20, 24, 25], as well as works studying the background electric activity of the spinal cord [5, 13, 17, 18, 19, 22, 26, 27, 28]. But these investigations also were carried out under experimental conditions differing greatly from the physiologic conditions, i. e. under conditions of operative exposure and sectioning of various portions of the central and peripheral nervous systems or with anesthesia or curarization.

We decided to experiment with the puncture method of leading off the bioelectric potentials of the spinal cord in a practically intact animal (rabbit) in order to approximate the physiologic conditions as nearly as possible.

EXPERIMENTAL METHODS

The experiments were performed on 19 rabbits. We describe the puncture method of leading off the bioelectric potentials of an animal's spinal cord in some detail because of the lack of literature available on the use of this method.

The anatomical peculiarities of the rabbit's spine [12, 14] makes it possible to puncture the spinal canal at two levels: at the inferior thoracic level – on either side of the spine of the XII thoracic vertebra, which is perpendicular to the spinal column (spines of the other thoracic vertebrae are caudally directed, while those of the lumbar vertebrae are cranially directed) and at the sacral level – at the point where the rabbit's sacrum has an unmatched foramen in its center, between the united I and II sacral vertebrae, which is about 2 mm in diameter, i. e. wide enough to permit the passage of a needle electrode.

The puncture lead was unipolar. The active electrode, a steel hypodermic needle, was inserted between the vertebrae D_{11} and D_{12} or between D_{12} and L_1 and into the foramen in the center of the sacrum between the vertebrae S_1 and S_2.

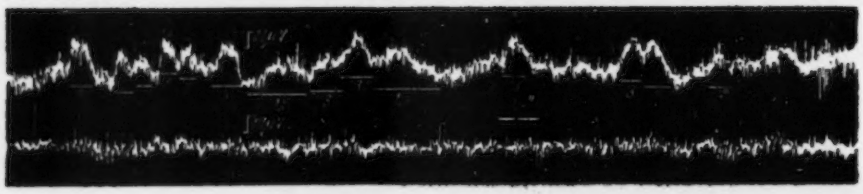

Fig. 1. ESG of the lumbosacral level (top curve) and corresponding EMG (bottom curve). Clearly marked, slow action potentials evident in ESG only. First type of electroactivity. The frequencies of the fluctuation periods are given in cps. Time indication = 0.5 second.

The animals were fastened to the usual laboratory bench. The needle seemed to drop at the moment at which it entered the spinal canal. At this moment, the rabbit manifested an acute, painful, motor reaction. The position of the active electrode in the cerebrospinal canal was checked by opening the region after the experiment.

We found that the tip of the electrode had passed through into the substance of the spinal cord at the thoracic level, while at the lumbosacral level, it was located among the cauda equina roots and reached into the conus medullaris.

Under these conditions, the leads recorded the overall bioelectric effect induced by the nervous processes in the zone of the lead, including the electric field changes connected with peculiarities of the electric processes taking place in the volumetric conductor [9, 16, 21 et al.].

The passive needle electrode was either inserted alongside into the paravertebral muscle or (more often) inserted into the same interspinal muscle as the active electrode, or into the interspinal muscle of the adjoining vertebra. There was a distance of 2-7 mm between the active and passive electrodes.

Of course, this method of leading off also recorded the biological currents of the muscles located between the electrodes. The use in several experiments of insulated (all but the tips) electrodes had no advantage over the use of non-insulated ones, because the muscle currents were led off by the passive electrode even with this insulation. Therefore, we used noninsulated electrodes as a rule.

This meant that the muscle (electromyogram – EMG) and cerebrospinal (electrospinogram – ESG) components of the overall bioelectric effect had to be distinguished from each other. This was accomplished by synchronously recording an EMG of the interspinal or paravertebral muscle through which the electrodes leading off the action potentials of the spinal cord passed. As we will show, comparison of the ESG with the EMG data made it possible to connect a series of bioelectric phenomena solely with the activity of the spinal cord.

In leading off the bioelectric potentials with needle electrodes, sufficient practice showed that the brief traumatic effect caused by the insertion of the leading electrodes could be disregarded if, as in our experiments, recording of the bioelectric potentials was started 15-30 minutes after the insertion of the electrodes and was continued for several hours. Moreover, the physiologic nature of the recording processes was indicated by the fact that the electric activity being recorded varied according to the changes induced in the functional condition of the substrate.

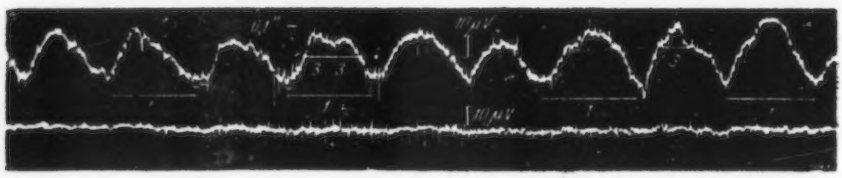

Fig. 2. Animal coming out of ether anesthesia. Slow waves in time with respiration apparent only on the ESG (Orbeli-Kunstman phenomenon; for original background, see Figure 1). Order and significance of curves the same as in Figure 1. Indication of time = 0.1 second.

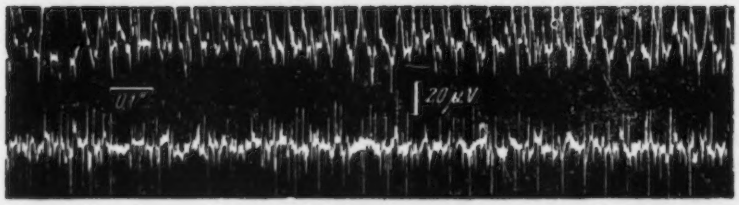

Fig. 3. The second type of electroactivity of the lumbosacral level. Uninterrupted slow waves can be seen with frequencies of 10 to 30 v/sec (amplitude 5-20 μ v) which raise in peaks or groups of peaks with amplitudes of 40-50 μ v or more.

The variations of the potential were recorded with a loop oscillograph, powered by a two-channel, AC amplifier (manufactured by the Experimental Factory of the AMN SSSR). Frequencies of 0.2-0.5 cps were distorted 12-18%. We used strong amplifications (usually 1 microvolt per 1-2 mm of ray deflection). Amplifier noise was 2-3 microvolts in value.

EXPERIMENTAL RESULTS

I. Comparing the variations of the potential recorded with the puncture lead from the spinal cord (ESG) with the curves of the action currents of the corresponding muscle (interspinal or paravertebral), we obtained arguments in proof of the fact that with the use of a puncture lead, the bioelectric currents of the spinal cord are led off along with the muscle action currents. These arguments are as follows:

1. When the frequency characteristics of the EMG and the ESG were compared, slow action potentials, which were not present in the electromyogram, were clearly evident in the ESG (Fig. 1). This was to be expected, since the electric activity of the nerve cells, which together make up the gray matter of the spinal cord, is expressed by relatively slow variations of the potential. Muscle electroactivity, however, reflects the advent in the muscle of spreading excitation waves and is characterized by action potentials of relatively high frequency.

2. In a series of experiments, we observed that the dynamics of the relatively fast activity differed in the ESG and EMG. The muscle component of the ESG reflected the activity of the same muscles recorded by the EMG; therefore, this difference could only be connected with the nonmuscle component of the EMG, i. e. with the activity of the actual spinal cord. For example, a high-amplitude, frequent discharge sometimes appeared first in the ESG and then appeared later in the corresponding EMG.

During ether anesthesia, there was a stage in which the frequent discharge disappeared in the EMG, but remained clearly evident, although diminished, in the spinal cord. At the first stage of curarization (a few minutes after the intramuscular injection of 15 mg of elatin – Delphinium elatum alkaloid, $C_{36}H_{50}O_9N_2$), the fast waves of the EMG were observed to be depressed, while this activity was still relatively high in the ESG.

3. Regularly, at a specific stage in anesthesia (sometimes even with a mild degree of curarization), slow action potentials in time with the respiration appeared in the ESG at the lumbrosacral level of the spinal cord, but were not recorded on the corresponding EMG (Fig. 2). These changes in the electric potentials could be interpreted as a specifically electrographic expression of the known Orbeli-Kunstman [10] phenomenon.*

All these facts indicate that with the method we used, the bioelectric activity of the spinal cord was recorded along with the muscular activity. The slow action potentials found in the ESG were the most carefully considered, as their cerebrospinal origin was certain, while the fast potential waves were taken into consideration only if there was evidence of their nonmuscular (spinal) origin.

II. We obtained the bioelectric characteristics of each of the two cerebrospinal levels from which the bioelectric potentials were recorded.

* The question of this phenomenon and the results obtained from research on the influence of induced changes in the functional condition of the central nervous system on background electroactivity will be taken up in detail in other articles.

1. At the lumbosacral level, the background activity observed could be reduced to two main types, which evidently reflected different functional conditions of the spinal nerve apparatuses, even though the recording was always done while the animal was outwardly immobile.

The first type of electroactivity was characterized by the presence of slow waves, singly or in small groups (see Fig. 1), with a period of 0.5-0.15 second, i. e., corresponding in frequency to the delta and theta EEG waves (2-6 waves per second). The voltage of these waves was usually 10-20 microvolts. Often, even slower fluctuations of the potential were observed, having a period of 1-4 seconds, or even sometimes 7 seconds, and approximately the same voltage. In one or two of the experiments, these very slow fluctuations of the potential were recorded as groups of waves. This type of activity was usually recorded from an animal which had been at rest for a prolonged period of time.

The second type of electroactivity was characterized by relatively high-amplitude, single or grouped peaks, with a slow wave at the base. These peaks (or groups of peaks) and waves proceeded rhythmically, with a frequency of 8-14 per second (like the alpha waves of the EEG) or 25-30 per second (like the beta waves of the EEG, Fig. 3). The peaks evidently reflected simultaneous discharges of the motoneurons, because they often appeared in the same rhythm on the EMG. Sometimes, the EMG also showed faint, slow waves, which seemed to be a faint repercussion of the slow waves shown on the ESG. It is possible that these waves appeared on the EMG in connection with the physical distribution of the spinal cord potentials, the EMG being recorded from the interspinal muscle of the same or adjoining spinal segment. The two types of activity were observed to alternate in the same animal. The second type of electric activity was recorded in the animal shortly after motor activity, but when the animal was in a resting condition. A high-frequency, low-amplitude (usually 5-10 microvolts), essentially the same as that recorded simultaneously on the EMG, was also invariably observed on the ESG of the lumbosacral level. We did not take this activity into consideration, although it could be proposed that it also contained a spinal component.

2. The slow fluctuations of the potential in time with the respiration were observed at the "higher", or inferior thoracic level. One could sometimes observe a single, high-frequency discharge on the ascending line and at the top of the wave. The nature of these action potentials will be thoroughly examined in a separate article. The ECG is almost always recorded at this level, and the activity at this level was essentially the same as that of the simultaneously recorded EMG.

The results we obtained with the use of the puncture method of recording the background electroactivity of the lumbar portion of a rabbit's spinal cord were very similar to those of other authors, but the features of this method enabled us also to obtain many new facts.

F. Bremer [19], recording the bioelectric potentials of a cat's spinal cord, observed slow, alpha-like waves along with the faster activity. In the detailed investigations conducted at the J. ten-Cate Laboratory on the background electroactivity of the spinal cord [27, 28], the presence of slow fluctuations resembling alpha, beta and delta waves was demonstrated. C. Ajmone-Marsam, M. Fuortes and F. Marosero [13] described slow fluctuations of the potential similar to alpha waves in the background electroactivity of a dog's spinal cord. Similar fluctuations were described by F. V. Bassin, B. P. Malkiel' and Iu. S. Iusevich [3], using a puncture method of leading off the bioelectric currents of the human spinal cord. A. Brandon [18], who investigated the electroactivity of a guinea pig's isolated spinal cord during anaphylactic shock, described the presence of slow waves with a period of varying length, as well as peaks with a slow wave at the base; this is very close to the second type of electric activity which we observed in the rabbit's spinal cord.

The phenomenologic similarity of the background electroactivity of the spinal cord, as recorded by the puncture method of leading off, to the results obtained in the works by the above-mentioned authors by methods leading off from the exposed spinal cord is a conclusive indication of the value of this lead method to research on the electric activity of the animal spinal cord. On the other hand, it also follows that many of the activity patterns of the spinal cord neurons, disclosed by methods leading off from an exposed (or even isolated) spinal cord also characterize spinal cord activity under physiologic conditions.

Due to the fact that we conducted our experiments under conditions more closely resembling the physiologic than those under which the other authors conducted their research, we obtained many new data. These include the differences observed in the electroactivity of the spinal cord at the thoracic and lumbar levels, the association of a specific type of electric activity with the level of activity, the presence of extremely slow

variations of the potential in the ESG, the electrographic expression of the Orbeli-Kunstman phenomenon and certain data obtained as a result of induced changes in the functional condition of various sections of the nervous system (see footnote, page 1069).

A few words concerning the extremely slow variations of the potential in the background electroactivity of the spinal cord. We used an AC amplifier, which means that the recording of these variations distorted them as to length, amplitude and possibly form [4]. However, the data obtained permit mention of the extremely slow variations of the potential in the spinal cord, although this data is not a reliable index of their true quantitative character (this can be determined by the use of a DC amplifier).

A. B. Kogan [8], A. Leao [23] and N. A. Aladzhalova [1, 2] demonstrated the presence of very slow action potentials in the brain, and N. A. Aladzhalova was able to prove the connection of these action potentials with the excitation waves of specific neuron structures in the brain. It is possible that the extremely slow variations of the potential we recorded were also associated with some kind of excitation waves produced by spinal cord elements. This, however, is a subject which needs further experimental development.

SUMMARY

A puncture method of leading off the biopotentials of rabbit's spinal cord is described. It gives an idea of the background electroactivity of the spinal cord in these animals in conditions maximally approaching physiological. Therefore, this method made it possible to obtain certain new data in addition to facts discovered by a number of research workers in conditions of spinal cord exposure.

The characteristics of the background electroactivity was determined at two levels where the potentials were led off: the inferior thoracic and the lumbosacral. Two types of electroactivity were revealed at the lumbosacral level. Each of these types were evidently connected with definite forms of activity of the neuron structures of the spinal cord. Very slow fluctuations of the potential (with the period ranging from one to several seconds) were noted in the electroactivity at this level.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

THE EFFECT OF REPEATED ADMINISTRATION OF SUBLETHAL DOSES OF TETANUS TOXIN ON THE ORGANISM

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E. Behring [8] noted that when horses were immunized with small doses of tetanus and diphtheria toxins, the animals died after administration of toxin in a dose much smaller than the lethal one. Sometimes the animals died when enough antitoxin to effect neutralization of the toxin was present in the blood.

Further investigations showed that development of increased sensitivity to small doses of bacterial toxins played an important role in the pathogenesis of toxic infections. S. M. Minervin and E. N. Kotliarovskaia [5] established the importance of sublethal doses of botulin toxin in the pathogenesis of botulism. The role of the Behring phenomenon in the pathogenesis of diphtheria intoxication was shown by A. T. Kravchenko and N. V. Galanova [2], as well as K. I. Matveev, T. I. Bulatova and A. P. Gindin [4] who observed death and development of pathomorphologic changes in the organs when guinea pigs were subjected to repeat administration of sublethal doses of diphtheria toxin. K. I. Matveev [3] obtained botulism development in animals which received daily injections of botulin toxin in doses amounting to hundredth and thousandth parts of the lethal dose. O. Ia. Ostryi, Z. I. Sobieva and A. N. Aliev [7] have shown experimentally that the process of summation of pathogenic nervous stimuli resulting from administration of subthreshold doses of toxins produced by gas gangrene pathogens can lead to a fatal outcome when the injections are given over microintervals of time.

In order to study the pathogenesis of tetanus we investigated the action of repeated administration of sublethal doses of tetanus toxin on the animal organism.

EXPERIMENTAL METHOD

Experiments were carried out on guinea pigs weighing 300-350 g, on mice weighing 16-18 g and on rabbits weighing 1200-1500 g. First the lethal dose of dry tetanus toxin was titrated for each species of animal on intradermal injection; it was 0.0005 mg for mice, 0.004 mg for guinea pigs and 0.05 mg for rabbits. During the experiments the weighed toxin was dissolved daily in a definite volume of physiologic solution to give a concentration of 1 Mld in 1 ml. After this one lethal dose was diluted 50, 100, 250, 500 and 1000 times. Various groups of animals were given daily 1/50, 1/100, 1/250, 1/500 and 1/1000 Mld subcutaneously in a hind limb or back. Antitetanus serum was used to treat some of the animals when they developed the disease. The activity of the tetanus toxin used in the experiments was controlled by daily titration using mice.

EXPERIMENTAL RESULTS

The first series of experiments was carried out on 67 guinea pigs of which 33 served as controls (see Table).

The animals which received 20/50, 3/100, 4/250, 4/500 and 6/1000 Mld of the toxin showed the first symptoms of tetanus on the 3rd-7th day. On further administration of the toxin a typical picture of local and then (on the 17th-21st day) of general tetanus was observed. As soon as the first symptoms of tetanus made their appearance the animals were given a toxin injection subcutaneously in the back in order not to traumatize

TABLE

The Effect of Sublethal Doses of Tetanus Toxin Administered Daily to Guinea Pigs

No. of experiments	Experiment (repeated administration of toxin)							Control (single administration of toxin)					
	No. of guinea pigs	Single dose of toxin (in Mld)	No. of toxin injections	Total amount of subcutaneously injected toxin	result: fatal, No.	result: fatal, Day on which death occurs	result: Recovery, no. of guinea pigs	No. of expt.	No. of guinea pigs	Single dose of toxin (in Mld)	result: fatal, no.	result: fatal, day of death	result: Recovery, no. of guinea pigs
1	4	1/50	20	20/50	—	—	4	1	3	20/50	—	—	3
2	6	1/100	12—19	12/100—19/100	3	12—19-й	3	2	3	7/100	—	—	3
3	5	1/100	10—13	10/100—13/100	5	10—13-й	—	3	3	3/250	—	—	3
4	3	1/250	30	30/250	3	30—31-й	—	4	3	4/500	—	—	3
5	4	1/250	15—24	15/250—24/250	4	15—24-й	—	5	3	20/1000	—	—	3
6	4	1/500	18—25	18/500—25/500	4	18—25-й	—	6	3	3/100	—	—	3
7	3	1/500	26—27	26/500—27/500	1	27-й	2	7	3	4/250	—	—	3
8	5	1/1000	19—20	19/1000—20/1000	5	19—20-й	—	8	3	4/500	—	—	3
								9	3	19/100	—	—	3
								10	3	30/250	—	—	3
								11	3	26/500	—	—	3
total	34				25		9		33	—	—	—	33

the leg in which the toxin was first injected. The animals died when the clinical picture of tetanus was pronounced. When sublethal doses of toxin were given daily the animals received a total of only 12/100-19/100, 24/250-30/250, 18/500-27/500, 19/1000-20/1000 Mld in the course of several days.

The clinical picture of tetanus was very characteristic in guinea pigs which received 1/1000 Mld toxin daily. Local tetanus of first degree appeared in this group on the 12th-14th day. Further injections of the toxin were made subcutaneously in the back. Local tetanus persisted until the 16th-17th day, then disappeared and kyphosis of the spine made its appearance. On the 21st-22nd day fulminating general tetanus developed and the animals died within 2-3 days. In rare cases the guinea pigs recovered.

Of 34 experimental guinea pigs 25 died from general tetanus, 9 animals had severe tetanus but recovered. Of 33 control animals which received the total dose in one injection, 18 animals remained quite healthy and 15 showed mild, transient manifestations of tetanus of first degree. Blood specimens taken on the 13th, 15th and 21st day from the beginning of toxin injections did not show even 1/1000 active units of antitoxin.

The second series of experiments was carried out on 20 mice and 6 rabbits. When the mice were given 1/25 and 1/50 Mld tetanus toxin daily they developed tetanus of the first degree on the 3rd-4th day. Further injections caused rapid development of general tetanus and all the mice died after receiving a total of only 6/25-7/50 Mld of toxin. Ten control mice which received the total dose in a single injection developed first degree tetanus but recovered after 9 days.

In I. N. Morgunov's and V. V. Khatuntsev's experiments [6] on mice the animals died from tetanus on daily injection of 1/10-1/25 Mld of tetanus toxin. Daily injection of 1/50 Mld of toxin led to the development of the clinical picture of tetanus but the animals did not die.

Six rabbits which received daily 1/1000 Mld toxin developed first degree tetanus on the 9th day; they were subsequently given the toxin subcutaneously in the back and by the 14th day they developed third degree tetanus. After 18 injections further administration of toxin was stopped since the rabbits began to recover. The rabbits were perfectly healthy after 39-40 days; no antitoxin was found in their blood.

The rabbits thus did not contract fatal tetanus when they were given sublethal doses of tetanus toxin.

Other investigations were devoted to antitetanus serum treatment of animals which contracted tetanus with daily injections of sublethal doses of tetanus toxin.

In 2 experiments 12 guinea pigs received 1/1000 Mld tetanus toxin daily. On the 21st day, i. e. after the appearance of general tetanus, they were treated. Each was given from 750 to 1000 active units of antitoxin serum subcutaneously. All the animals died from tetanus within the same periods of time as those which had not had serum treatment. In the third experiment 6 guinea pigs received 1/250 Mld toxin every day. On the 5th day from the start of injections, i. e. prior to the appearance of the signs and symptoms of tetanus, 3 animals were given 750 active units of serum and 3 animals were left untreated. The animals which had received the serum remained healthy, while those which had not been given the serum died from tetanus on the 15th-17th day.

These experiments show that the therapeutic action of the serum depends, with daily injections of sublethal doses of tetanus toxin, on the time of administration. Early administration of serum, prior to the appearance of tetanus manifestations, protects the animals from death; late administration of serum has no therapeutic effect and the animals die from tetanus. A similar picture is seen when serum therapy is used for man and animals following tetanus infection under natural conditions (wounds).

The investigations carried out suggest that when tetanus is contracted by man or animals following wounding the tetanus pathogen undergoes gradual multiplication in the wound during the incubation period and produces small, sublethal doses of toxin, without an immediate effect on the organism. The length of the incubation period depends on the amount of toxin produced by the pathogen and on the resistance of the organism. Consequently in cases of tetanus following wounding gradual action of sublethal doses of toxin takes place and leads to the development of the disease and finally to a fatal outcome.

I. N. Morgunov and V. V. Khatuntsev [6] have shown that the rate of development of the disease is directly related to the magnitude of the single dose of toxin and inversely proportional to the intervals of time between the appearance of the toxin.

P. F. Zdrodovskii [1] explains the increased sensitivity to repeated administration of sublethal doses of bacterial toxins by summation of stimulation, drawing on electrophysiology for this concept.

Since repeated administration of small doses of tetanus toxin to animals leads to gradual development of the clinical picture of tetanus similar to that seen with wound tetanus, with impairment of important vital processes in the nervous and muscular systems, we consider that this phenomenon represents not summation of stimuli but a summation of lesions. The gradual action of very small doses of tetanus toxin on the organism also suggests that it may be an antimetabolite subserving impairment of enzymatic processes in the nervous, muscular and other tissues of the body, with no clearly defined histologic changes in these tissues.

SUMMARY

When tetanus toxin is repeatedly administered to guinea pigs and mice in sublethal doses it causes an attack of tetanus resulting in death. This takes place when the sum total dose of administered toxin is considerably lower than the lethal dose. In single administration of the whole dose of the toxin (used formerly in several injections) the animals remain healthy or sustain an attack of tetanus from which they soon recover. The therapeutic serum was found to be ineffective in late treatment of tetanus which developed as a result of daily administration of sublethal doses of the toxin to guinea pigs.

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THE ROLE OF LIVER IN THE MECHANISM OF HYPOGLYCEMIC ACTION OF THE ANTIDIABETIC SULFANILAMIDES•

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It was shown in a previous communication, on the evidence of both in vitro and in vivo experiments, that the hypoglycemic effect of the antidiabetic preparation BZ-55 (nadizan, N-sulfanilyl-N^1-butyl-carbamide) was due to its inhibition of liver insulinase (an insulin-splitting enzymatic complex). The effect of this drug could not be demonstrated at all in depancreatized animals, while in alloxan-diabetic animals it was only effective if some residual insulin synthesizing capacity was retained in the pancreas. It was also found that, both in normal and in depancreatized animals, nadizan enhanced the hypoglycemic effect of introduced extraneous insulin. It was thus concluded that the hypoglycemic effect of the various sulfanilamide preparations could only take place when insulin, whether endogenous or exogenous, was actually present within the body.

However, a number of problems still remained unsolved: 1) did nadizan, as claimed by some authors [1, 5], exert any stimulating effect on the β-cells of the islet tissue of the pancreas; and, 2) was the enhancement by nadizan of the hypoglycemic action of insulin due entirely to its inhibitory effect upon insulinase, and consequently to the preservation of small amounts of the hormone from destruction, or was there also a specific peripheral activation of insulin by the drug.

The problem was thought capable of solution only under conditions where the islet tissue function was preserved completely, while liver insulinase was ineffective. If under such conditions nadizan should fail to exert its hypoglycemic effect, or to activate introduced extraneous insulin, it could be taken as evidence that the effect of the drug involved neither the islet tissue, nor the peripheral action of insulin itself, and that its hypoglycemic effect was due purely to insulinase inhibition. The present work was based on this theory.

EXPERIMENTAL METHODS

Liver damage was caused in experimental rabbits through subcutaneous injection of CCl_4 (1 ml/kg body weight, 3-5 injections at 48-hour intervals). Gross fatty infiltration was taken as a criterium of liver damage (increase in fat content, on dry weight, to 27-37%; normal value – 16.6 ±1.52%). The glycemic reaction to insulin (0.1 unit/kg), nadizan (0.3 kg) and the two factors given simultaneously was determined in all animals before and after poisoning. Liver insulinase activity was determined in both normal and poisoned animals by the method of Mirsky [3], and of Sklovskaia and Vol'kenzon [2], as follows. The livers were excised and placed into a Petri dish cooled in ice, and ice-cold 1% sodium chloride solution was perfused through a canula placed into the hepatic artery until the perfusate emerged clear. Portions of the washed liver, weighing 20-22 g, were chopped up and ground with an equal volume of cold physiological saline solution in the presence of quartz sand. The homogenate was pressed through cloth, the exudate was centrifuged and the filtered supernatant was dialyzed for 18 hours against distilled water. At the end of the dialysis period the precipitate was centrifuged off and the supernatant filtered. The filtrate thus obtained contained the albumin fraction of

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TABLE 1

The Effect of CCl_4-induced Liver Damage on Insulinase Activity and the Glycemic Reaction to Insulin

Rabbit No.	CCl_4 dose (in ml/kg) × number of doses	Liver insulase activity (% decrease of initial glycemic		Glycemic reaction to the introduction of 0.1 unit of insulin/kg before CCl_4-poisoning				Glycemic reaction to the introduction of 0.1 unit of insulin/kg after CCl_4-poisoning				Liver fat, g%
		untreated extract	inactivated extract	initial blood sugar level, mg%	blood sugar level, Mg% at 1.5 hours	blood sugar level, Mg% 3 hrs.	maximal fall, in % of initial level	initial blood sugar level, mg%	blood sugar level, Mg% at 1.5 hours	blood sugar level, Mg% 3 hrs.	maximal fall, in % of initial level	
1	1×5	32.0	38.5	—	—	—	—	—	—	—	—	—
2	1×3	21.7	38.4	—	—	—	—	—	—	—	—	—
3	1×4	26.0	32.4	—	—	—	—	—	—	—	—	—
4	1×3	28.1	42.2	122	108	83	31.9	117	77	66	43.6	27.7
5	1×4	33.0	40.9	125	93	90	28.0	108	65	56	48.2	34.2
6	1×5	27.7	29.2	127	91	99	28.3	129	75	65	49.5	37.5
7	1×3	29.0	28.3	108	84	83	23.1	108	65	56	48.2	33.5
8	1×3	—	—	110	84	84	23.6	134	66	79	50.7	—
9	1×3	31.1	32.4	112	108	83	23.7	125	86	72	42.4	27.5
10	1×4	31.1	30.5	140	122	98	30.0	99	75	50	49.4	28.0
11	1×3	36.4	41.2	141	113	97	31.2	136	82	60	56.6	32.5
12	1×3	33.3	46.1	108	87	80	25.9	110	79	54	50.9	30.5
Mean		30.0	36.4	121	99	86	27.3	118	83	62	48.7	31.4

liver proteins, but not the low-molecular weight compounds like glutathione and cysteine, which are also capable of inactivating insulin; the extract was divided into two portions. Crystalline insulin, in amounts 1.5 units per 1 ml, was added to the first portion, the pH of the mixture was adjusted to 6.8 by the addition of the appropriate amount of Na_2HPO_4, and the whole incubated for one hour at 38°. The second portion was similarly treated, with the exception that the extract was inactivated by heating on the water bath for 30 minutes at 65-70°, before the addition of insulin. Insulin inactivation (and consequently, insulinase activity), was determined from the residual effect of the incubation mixture on the blood sugar of experimental rabbits. The incubation mixtures were given to rabbits subcutaneously, the dose being equivalent to 0.8 units of insulin initially present per kg. Blood samples were taken immediately before introduction of the reaction mixture and thereafter 1.5 and 3 hours after injection. Nadizan for intravenous injection was prepared as a 2.5% solution of the pure substance in an alkaline solution (pH 8) of sodium bicarbonate.

EXPERIMENTAL RESULTS

It was shown in the previous communication [1] that, while the introduction to rabbits of insulin previously incubated with inactivated dialyzed liver extracts caused a mean decrease in blood sugar of 30%, insulin incubated with intact liver extracts produced decrease in blood sugar of up to only 16% (mean value, 6%). It was thus possible to conclude, in agreement with Mirsky [3, 4], that normal rabbit liver contained an insulin-destroying enzymatic complex, which Mirsky termed insulinase.

It will be seen from the data presented in Table 1 that, in the majority of cases, the untreated extracts obtained from CCl_4-poisoned rabbits, when incubated with insulin and injected into test animals, produced an effect similar to that obtained with the inactivated normal liver extracts (the mean fall in blood sugar in the former case was 36.4%, in the latter — 30%). Thus, during fatty degeneration caused by CCl_4 poisoning, the liver was unable to destroy insulin, that is, the enzymatic complex insulinase was either absent from livers in

TABLE 2

The Effect of CCl_4-induced Liver Damage on the Hypoglycemic Activity of Insulin and Nadizan

Rabbit No.	Initial blood sugar level	Introduction of insulin	Blood sugar levels in mg%, before CCl_4 introduction, after an interval of				maximal decrease in % of initial levels	CCl_4 dose (in ml/kg) X number of doses	initial blood sugar level	Blood sugar levels in mg%, before CCl_4 introduction after an interval of				maximal decrease in % of initial levels
			15 min.	30 min.	1 hour	2 hours				15 min.	30 min.	1 hour	2 hours	
1	117	—0.1 units/kg . .	102	92	88	84	28.2	1×3	141	106	83	77	75	46.6
	119	—0.3/ kg	97	83	83	90	30.2	1×3	110	108	104	106	99	10.0
	127	—0.1 units/kg + —0.3/ kg	104	79	68	61	51.9	1×3	125	102	79	77	72	42.4
2	111	—0.1 units/kg . .	92	84	83	83	25.2	1×3	106	81	61	52	66	50.9
	120	—0.3/kg	93	79	68	63	47.5	1×3	113	113	115	108	104	7.9
	106	—0.1 units/kg + —0.3/ kg . . .	70	65	57	48	54.6	1×3	124	97	86	72	63	49.2
3	101	—0.1 units/ kg . .	90	79	81	79	21.7	1×3	122	84	72	70	61	50.0
	124	—0.3/ kg	93	79	68	70	45.1	1×3	115	113	117	110	104	9.5
	106	—0.1 units/ kg . .	70	65	57	48	54.6	1×3						
	115	—0.1 units/ kg + —0.3/ kg. .	93	77	65	54	53.0	1×3	104	79	68	57	66	54.7
6	119	—0.1 units/ kg . .	92	79	84	88	25.1	1×3	90	70	61	59	50	44.4
	107	—0.3/ kg.	80	69	63	60	49.3	1×3	83	83	75	74	81	10.8
	125	—0.1 units/kg + —0.3/ kg. . .	83	66	47	52	62.4	1×3	97	86	50	52	45	55.0
7	95	—0.1 units/ kg. . .	77	68	66	63	33.6	1×3	106	90	68	63	52	50.9
	86	—0.3/ kg.	65	54	57	53	37.2	1×3	105	100	100	93	93	11.4
	99	—0.1 units/ kg + —0.3/ kg . . .	75	50	56	48	51.5	1×3	104	79	68	59	56	46.2
8	103	—0.1 units/kg . .	86	78	77	81	21.3	1×3	103	90	63	47	62	54.3
	110	—0.3/ kg	102	97	87	74	32.7	1×3	119	112	112	111	114	6.7
	118	—0.1 units/ kg + —0.3/kg . .	99	83	75	68	42.4	1×3	115	76	61	53	61	53.9
4	102	—0.1 units/kg . .	81	77	79	77	24.5	1×3	97	81	68	47	50	51.4
	120	—0.3/ kg	83	81	68	66	45.0	1×3	113	110	111	106	102	9.7
5	95	—0.1 units /kg . .	74	73	74	70	26.3	1×3	88	74	57	47	7	47.1
	106	—0.3/ kg	84	75	61	65	42.4	1×3	92	92	90	84	88	13.5

this pathological state or, if present, had a very low activity.

It may be expected that the depression of insulinase activity, observed in the damaged liver, would result in a higher overall hypoglycemic effect of insulin, when given to poisoned animals. As seen from Table 1, this was actually the case; in all experiments, the single standard dose of insulin (0.1 unit/kg) was more effective in the animals suffering from carbon tetrachloride poisoning, than it was in the same animals before the administration of CCl_4 The mean fall in blood sugar caused by this dose in normal animals was 27.3%, while after CCl_4 poisoning the corresponding value rose to 48.7%.

In normal rabbits, prior to CCl_4 poisoning, nadizan given intravenously caused, within two hours, a fall in blood sugar of 30-45% (mean value, 40%); in CCl_4-poisoned animals the same dose of nadizan produced a fall in blood sugar of only 6.7-13.5% (mean value, 9.9%), that is, one within the limits of so-called spontaneous variations in blood sugar expected in any 2-hour period (Table 2).

Thus, during liver damage and the concomitant inactivation of insulinase, nadizan showed no hypoglycemic effect, even though the function and structure of the islet tissue remained intact; the reactivity to introduced insulin actually increased. In CCl_4-poisoned animals nadizan also failed to enhance the activity of added insulin, an effect invariably produced in these animals prior to poisoning.

Dulin and Johnston [6], using hepatectomized rats and dogs, maintained on infused glucose, observed a fall in blood sugar in these animals after introduction of tolbutamide (D-860) directly after operation. This effect was attributed to a stimulation of insulin secretion. It should, however, be borne in mind that, under such extremely un-physiological conditions, tolbutamide may have had a non-specific effect on blood sugar levels, particularly since hepatectomized animals develop hypoglycemia, at which stage glucose infusion stimulates insulin secretion. The present authors consider the experiments described above as providing a more satisfactory understanding of the role of liver in the hypoglycemic action of sulfonamides.

The present results thus established that under conditions of liver damage which lead to insulinase inactivation, nadizan had no hypoglycemic effect, nor did it enhance the effect of added insulin. The data show that nadizan has no effect on the peripheral action of insulin. The hypoglycemic effect of this drug is due to its inhibitory action on liver insulinase.

SUMMARY

Preparation BZ-55 caused hypoglycemia in normal rabbits, potentiated the hypoglycemic effect of insulin and inactivated insulinase of the liver. Insulinase is not found in rabbits with fatty dystrophy of the liver caused by carbon tetrachloride intoxication. The hypoglycemic effect of insulin is increased. Administration of BZ-55 (Nadizan) does not cause any hypoglycemic action and does not potentiate the effect of injected insulin, although the insular pancreatic apparatus is uninjured. These experiments permit to draw a conclusion that the hypoglycemic effect of BZ-55 is connected not only with the stimulation of the β-cells but also with the activation of the peripheral action of insulin. It is mainly caused by the inactivation of hepatic insulinase.

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CERTAIN DATA RELATING TO THE MECHANISM OF SECRETION AND ACTION OF ADRENOCORTICOTROPHIC HORMONE (ACTH) AND OF CORTISONE IN EXPERIMENTAL INFLAMMATORY CONDITIONS

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Numerous papers have appeared in recent years, reporting the successful use of hypophysial extracts, adrenocorticotrophic hormone (ACTH), or cortisone in the treatment of inflammatory processes, both in experimental animals and in patients [1, 4, 20, 22]. The mechanism of action of ACTH and of corticosteroids has not yet, however, been elucidated. The antiedema effect of ACTH and cortisone is ascribed to their lowering of the permeability of the walls of the blood vessels, due to changes in the function of the sympathetic nervous system [15], and to lowering of the levels of blood hyaluronidase and histamine [16]. Increase in the content of plasma proteins due to treatment with ACTH has been reported [2], and this may also contribute to abolition of edema. There is no unanimity of opinion as to the mechanism of enhancement of the adrenocorticotrophic function of the hypophysis. According to some workers, the secretion of ACTH in response to trauma is regulated by both reflex and humoral pathways [3, 7, 14, 21]; in this relation, N. V. Mikhailova [10], I. I. Rusetskii [11], and others stress the dominating role of the nervous system. On the other hand, certain authors [17, 19, 22] believe that regulation of the adrenocorticotrophic function of the hypophysis can be achieved by humoral factors alone, without the participation of the nervous system.

The contradictory nature of the published material cited above led us to undertake the present research. Its object was to elucidate the effects of nervous and humoral factors on secretion of ACTH by rabbits suffering from inflammation due to thermal injury, and also to investigate the effect of ACTH on the course of inflammatory conditions of the ear under conditions of intact innervation and of desympathization.

EXPERIMENTAL METHOD

Our animal material consisted of grey rabbits, weighing from 2 to 2.5 kg. Thermal injury was inflicted by dipping an ear in water at 80° for 3 seconds. We performed 5 series of experiments: 1st – control series; 2nd series – scalding after division of the sensory nerves (Auricularis major et posterior); 3rd series – scalding after exclusion of the sympathetic nerve supply of the ear; 4th series – treatment of the scald with ACTH (intramuscular injection of 1 unit per kg every 6 hours for 7-10 days); 5th series – ACTH treatment (as in the 4th series) of scalds of the desympathized ear.

The following observations were made over a period of 6-15 days after infliction of the injury: size of swelling, temperature of the site of inflammation and rectum eosinophile count [by N. V. Mikhailova's method [10]] and leucocyte count of venous blood taken from the unaffected ear, and phagocytic activity of the leucocytes towards killed Staphylococcus aureus. The eosinophile counts were made 1½ hours after taking the blood samples up into the mixing pipet. Eosinopenia was regarded as indicating activation of the function of the hypophysis – adrenal cortex system [22 and others]. We also recorded the time required for formation of a dry scab, and the duration of the inflammatory process (to when the scab was shed). The extent of necrosis was expressed as percentage of necrotic surface, in relation to the total surface of the scald. After detachment of the scab,

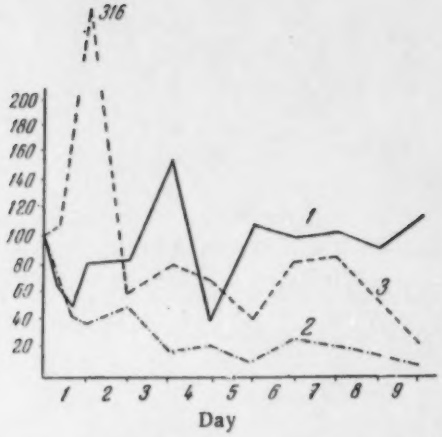

Fig. 1. Variations in the percentage content of eosinophiles in the peripheral blood of rabbits after infliction of thermal injury (mean values for each series)
1) Changes in percentage content of eosinophiles in the control experiments; 2) the same, for desympathized animals; 3) the same, for experiments involving deafferentation; ordinates — percentage content of eosinophiles in peripheral blood (the initial level is taken as 100); abscissae — days after infliction of injury.

we assessed the resistance of the animals to fatal scalding. This was done by pouring 1 liter of water at 100° on each of the hind legs, and then recording survival time.

EXPERIMENTAL RESULTS

Triggering mechanism for enhancement of adrenocorticotrophic function of the hypophysis in inflammation following thermal injury. As was mentioned above, a number of authors have shown that enhancement of the adrenocorticotrophic function of the hypophysis leads to a fall in the eosinophile count of the peripheral blood. Numerous authors [10, and others] take as evidence of increased secretion of cortisone the fall in the eosinophile count of peripheral blood, rather than their percentage content. In our opinion this procedure may be misleading, inasmuch as it is known that a number of pathological processes (including burns) are inaugurated by release into the circulation of blood having a high content of formed elements, from body depots. As a result, the eosinophile count rises, without much change in their percentage content. It is obvious that under such conditions it is possible to encounter an increase in the eosinophile count at the onset of a pathological state, instead of an eosinopenia, even although the level of corticoadrenal function has been raised; in such cases only a fall in percentage eosinophile content would reveal an early reaction to thermal trauma. For this reason we based our analysis of changes in adrenocorticotrophic function of the hypophysis on changes in the percentage content of eosinophiles in peripheral blood (Fig. 1).

We found in our control experiments that enhancement of corticoadrenal function took place in two episodes, as shown by eosinopenic episodes on the first and on some subsequent day of the experiments. In most of the experiments involving deafferentation we found an increase in the percentage content of eosinophiles, rather than an eosinopenia. This phase was then succeeded by a more pronounced eosinopenia than in the control experiments. At the same time, we observed more extensive necrosis in this series than in the controls, the necrotic area being 8% greater. Eosinopenia also proceeded biphasically in the experiments involving desympathization of the ear, the effects here being more pronounced than in the control series. The correlation between the degree of eosinopenia and the extent of the necrotic area was clearly evinced as the inflammatory process developed. In the experiments of this series the inflammatory process was associated with considerable edema (Fig. 2), and proceeded to necrosis of 94% of the scalded area (viz., 11% more than in the control series). Data relating to the duration of the inflammatory process, to the dimensions of the necrotic area, and to incidence of suppuration are recorded in Table 1.

Thus the initial rise in adrenocorticotrophic function of the hypophysis seen after scalding of intact and desympathized ears was absent when the afferent nerves of the ear had been divided. In the second phase, the degree of eosinopenia varies parallel with the intensity of the inflammatory process, and with the extent of the area of necrosis, irrespective of whether the afferent nerves of the affected region were divided or intact. These findings provide evidence, in our opinion, that in the first phase the mechanism triggering off enhancement of adrenocorticotrophic function of the hypophysis is preponderatingly of a reflex nature (stimulation of receptors in the inflammatory site), whereas in the second phase it is chiefly of a humoral nature (passage of breakdown products from the site of injury to the blood stream).*

* Analogous findings were obtained by us in recent experiments on white rats.

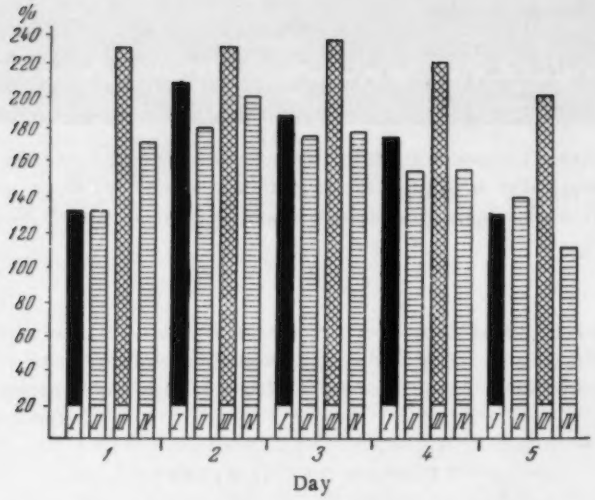

Fig. 2. Changes in the volume of rabbits' ears after scalding (mean results for each series of animals).
I) control series; II) experiments in which the animals received treatment for the burns; III) experiments in which the sympathetic nerve supply to the ear had been cut before scalding; IV) the same, with ACTH treatment; ordinates – volume of the affected ear, as percent of the initial volume, taken as 100%; abscissae – days after infliction of injury.

Effect of ACTH on the course of the inflammatory process. We found that the rise in temperature of the inflamed region was greater in animals treated with ACTH than in the control series: the temperature of the scalded ear exceeded that of the intact one by 1.8 – 4.4°, as compared with a difference of 1.1 – 2.9° found in the control series. This is evidence of the smaller disturbances in the local circulation [9], and confirms published reports of the intensification of metabolic processes caused by ACTH and cortisone [3, 18]. The ear was less swollen, from the first day of treatment (see Fig. 2). Similar findings have been reported by some authors [for example, [6, 15]]. Alleviation of the febrile condition reported by other authors to result from ACTH treatment [12] was not observed in our experiments. We found a greater leucocytosis in this series throughout the period of observation than in the control series. The phagocytary activity of the leucocytes rose steadily, although slightly (Table 2), which is in accordance with the findings of other workers [6].

TABLE 1

Duration of the Inflammatory Process, Extent of the Necrotic Area, and Frequency of Suppurative Complications of Scalds of Rabbits' Ears

Series	Day on which a dry scab formed	Duration of inflammatory process, in days	Extent of the necrotic area as % of total no. of expts.	Frequency of suppurative complications as % of the total no. of expts.
	2	3	4	5
1-	9-	22	83	40
2-	8-	23	91	20
3-	12-	32	94	90
4-	7-	24	79	—
5-	6-	21	96	—

Note: The figures under columns 2, 3 and 4 are mean values for a given series.

The fall in the percentage content of eosinophiles was more pronounced than in the control experiments. We found, however, that there was a transient rise in the eosinophile content of the peripheral blood on the 2nd-3rd day, in all the experiments. A dry scab was formed 2 days earlier, but it was shed 2 days later, than in the control series (see Table 1). The period of healing under the scab was thus prolonged by 4 days, which is evidence of retardation of proliferative processes due to the action of ACTH. In no case did suppuration supervene.

We thus found that ACTH treatment reduced the swelling and circulatory disturbances at the site of inflammation as compared with the control animals; the

TABLE 2

Phagocytic Activity of Rabbit Leucocytes after Thermal Injury.

Series	Day of experiment			
	1	2	4	6
1	2.9	2.4	1.6	2.7
4	1.6	1.7	1.8	1.7

Note: The initial level of phagocytic activity of the leucocytes is taken as unity. The figures given are the mean values per series.

extent of necrosis was also smaller. An unfavorable aspect of ACTH treatment is its depressing effect on the phagocytic activity of leucocytes, as well as its retarding effect on proliferative processes. Apart from these drawbacks, ACTH treatment had no effect on the febrile symptoms, and it caused an increase in the leucocyte count, as compared with the control series, a transient eosinophilia appearing on the 2nd-3rd days. These side-effects of ACTH treatment may have been due to the high content of protein contaminants in our preparations; this possibility, however, requires experimental confirmation.

Dependence of the therapeutic effect of ACTH on the sympathetic innervation of the site of inflammation. In the 5th series of experiments we applied ACTH to the treatment of burns of desympathized ears. We found that the temperature of the inflamed parts of the ear exceeded that found without treatment (3rd series): the difference between the temperature of the scalded and the intact ears varied from 2.6 to 4.5°, as compared with a range of 1.3-3.5° for the animals of the 3rd series. The ears were less swollen than for the animals of the 3rd series, or even of the control series, although more so than for the animals of the 4th series, in which ACTH treatment was given to rabbits with intact innervation of the ear (see Fig. 2). These findings are evidence that ACTH has a suppressive effect on edema of tissues deprived of their sympathetic supply. This result is not in accordance with N. P. Smirnov's interesting findings [15] that the sympathetic nervous system is involved in the lowering of capillary permeability taking place after administration of ACTH. It is possible that desympathized structures become more sensitive to the action of hormones, and not only to the corresponding mediators, as was shown by W. Cannon and A. Rosenblueth [5].

Suppuration was not encountered in this series, although in the 3rd series (untreated scald of a desympathized ear) it occurred in 90% of the cases. Finally, ACTH treatment of scalded desympathized tissues greatly shortened the duration of the inflammatory process, although the extent of the necrotic area was no smaller than in the experiments of the 3rd series. This finding points to the importance of the trophic function of the nervous system for the resolution of the inflammatory process; when this function is impaired treatment designed to alleviate secondary alterative changes (improvement of local circulation mediated by ACTH) are not effective in restricting the extent of the necrotic area.

Some data on the significance of the hypophysial-corticoadrenal system in the elaboration of immunity to the effects of burns. The possibility of developing immunity to the effects of burns has been demonstrated by the work of S. V. Skurkovich [13] and others. The results of our experiments confirm this finding. Whereas scalding of the hind legs of previously untreated animals caused their death in every case, with a mean survival time of 58 hours, of 8 rabbits which had recovered from scalding of an ear, without being given ACTH, two survived, and the mean survival time of the remaining 6 was 71 hours. Even greater resistance to lethal scalding was found for animals which had been treated with ACTH after scalding one ear; of 10 rabbits with scalded hind legs three survived, and the mean survival time of the remaining seven was 81 hours.

Our findings testify to the active participation of the hypophysial-corticoadrenal system in raising the resistance of animals to lethal scalding after recovery from minor burns.

SUMMARY

The course of the inflammatory process following scalding of one ear of rabbits, with and without previous deafferentation and desympathization of the ear, and with and without ACTH treatment, has been studied. The response of the adrenocorticotrophic function of the hypophysis proceeds biphasically, being triggered off by reflex action in the first phase, and by humoral action in the second. The temperature of the inflamed area is raised during ACTH therapy, while the swelling and the extent of the necrotic area are diminished and the incidence of suppuration is greatly reduced. With previous severance of sympathetic connections, ACTH treatment is also associated with higher temperature of the scalded region, edema is reduced, and the duration of the inflammatory process greatly shortened. The amount of necrosis is not, however, decreased. Rabbits which

have recovered from a minor burn are subsequently more resistant to major thermal injuries. The hypophysial-corticoadrenal system is actively involved in such raising of resistivity to thermal injury.

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THE REFLEX NATURE OF THE PHENOMENON OF "LAGGING" OF RESPIRATORY MOVEMENTS IN UNILATERAL LUNG LESIONS

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One of the clinical signs of unilateral lung lesions – abscess, atelectasis, pneumonia, etc. – is the so-called "lagging" of the chest cage on the side of the lesion, i. e. the slowing down of respiratory movements and diminution in their amplitude, most marked in the region of the lower ribs.

Such disturbances of function of the respiratory musculature appear to be reflex in nature, as supposed by the majority of clinicians (M. P. Konchalovskii, E. M. Tareev, E. M. Gelshtein, V. F. Zelenin, Stehelin and many others), indicating that when lobar pneumonia, pleurisy and similar diseases are encountered in clinical practice it is difficult to exclude the possibility of involvement of the diaphragm itself by a secondary inflammatory process.

Focal lesions in the lungs of animals can be produced experimentally without simultaneous inflammatory involvement of the diaphragm. However, the phenomenon of "lagging" cannot always be visually demonstrated even in the presence of extensive involvement of the lungs because of the lesser mobility of the chest cage in animals in which the shoulder girdle performs a supporting function [5]. For this reason we considered it expedient to investigate changes in respiratory movements employing electromyographic analysis of diaphragmatic contractions on the side of lung involvement.

EXPERIMENTAL METHODS AND RESULTS

Experiments were carried out on unanesthetized rabbits with laminar electrodes implanted onto the diaphragm. Biological currents were recorded using an encephalographic amplifier made by the Experimental factory of the AMN SSSR and an oscillograph MPO – 2.

Focal injury to the lungs was produced by the introduction into the lung through the chest wall of 2 ml of hot water (90°). In view of the fact that animals withstand poorly the bilateral suturing of electrodes to the diaphragm, experiments were run in two series: in one series the movement of the diaphragm on the side of the injury was recorded, in the other – on the opposite side. In connection with recording biological currents using laminar electrodes which permit taking into account only total changes in the activity of a given muscle segment, this investigation was limited to the study of changes in the amplitude of impulses. When amplification was used during quiet respiration the base line of the discharge on inspiration consisted of impulses of the order of 20 – 30 μ v and synchronized impulses of the order of 40 – 50 μ v.

In the presence of limited injury to the lungs, changes in the biological currents in the diaphragm follow an established pattern. Immediately upon the introduction of hot water an uninterrupted impulse with amplitudes 4-5 times greater than initially was observed. This reaction is more pronounced on the side of the injury than on the opposite side on which ordinarily there is a greater degree of preservation of the phase of increase and decrease of the impulse amplitudes corresponding to inspiration and expiration.

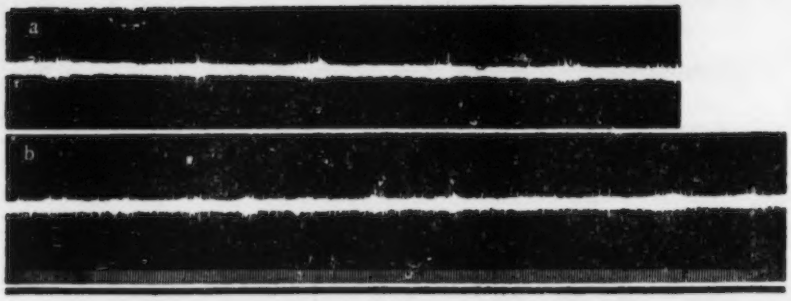

Fig. 1. Reaction of a rabbit diaphragm to injury of the homolateral lung. Electromyograms of the diaphragm: a) initially; b) several seconds after lung injury (diaphragmatic activity does not exceed the initial level despite considerable increase in respiratory rate). Amplification is 50 μv = 5 mm.

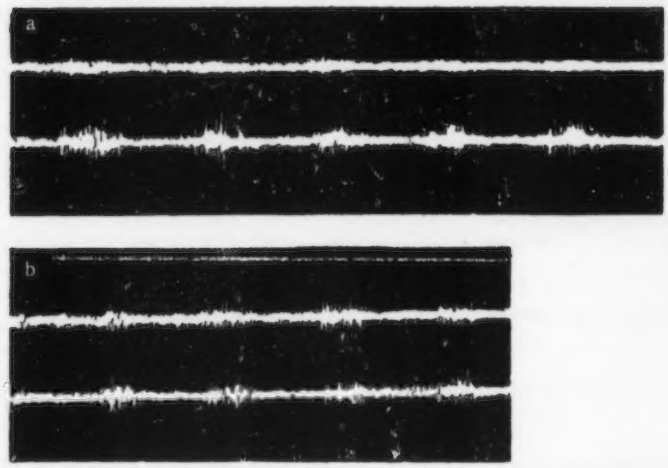

Fig. 2. Difference in reactions of the diaphragm and nasal muscles to lung injury. Tracings (from above down): electromyogram of alae nasi, electromyogram of the diaphragm.
a) Initially; b) 2 minutes after injury of homolateral lung (diaphragmatic activity less than initially, activity of nasal muscles is considerably higher). The recording rate is 3 cm/sec, amplification 50 μv = 5 mm.

On the side of injury the diaphragmatic impulse returns to its initial size in 1-2 minutes, and at times in several seconds, following lung injury, despite the persistent rise in the respiratory rate (Fig. 1). The more common impulses of lesser amplitude return to normal first and those of higher amplitude follow.

In addition, during the next 2-5 minutes the electrical activity of the diaphragm on the side of the lung lesion further weakens in most of the experiments, frequently dropping below the initial level, which is manifested in decrease in the amplitudes of the frequent impulses as well as in the almost total disappearance of high-amplitude impulses (Fig. 2). On repeated injury to the same lung this weakening of activity of the diaphragm lasted much longer (provided that the injected fluid was always introduced into lung parenchyma and not into a bronchus).

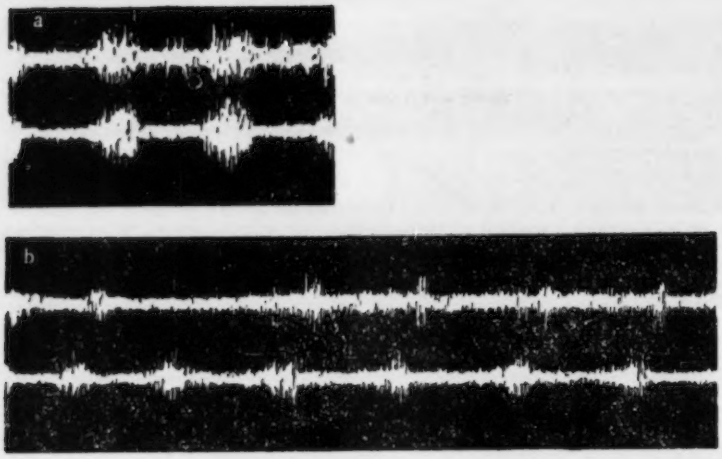

Fig. 3. Reaction of a rabbit diaphragm to injury of the contralateral lung. Tracings (from above down): electromyogram of alae nasi, electromyogram of the diaphragm.
Lower trading – initially; upper – 8 minutes after lung injury (diaphragmatic activity significantly increased). The recording rate is 3 cm/sec, amplification 100 μv = 11 mm.

On the contralateral side a rise in activity of the diaphragm, rather than a fall, was observed (Fig. 3) which lasted not less than 4-5 minutes following a single injury and considerably longer (from 15-20 minutes to several hours) following repeated injuries. This reaction could be abolished by injury to the lung of the same side, and conversely, weakening in activity of the diaphragm could be replaced with strengthening upon additional injury to the lung of the opposite side.

It must be pointed out that the phenomenon of "lagging" is associated not only with a decrease in amplitude of the respiratory movements on the side of the injury, but also with their slowing down, which at times can create the impression of paradoxical movement of the diaphragm (rise on inspiration and fall on expiration). However, some roentgenologists already turned their attention to the phenomenon that paradoxical movements of the diaphragm are possible only when the phrenic nerve is divided or paralyzed or when the lesion is in the diaphragm itself; in the presence of lesions in the lung, one observes only prolongation of the active phases of inspiration and expiration due to slowing down of the contraction of muscle [2, 4, 6, 8].

In experiments on rabbits this phenomenon is difficult to establish because of considerable activity of the diaphragm between inspirations which makes evaluation of the duration of discharge difficult. Preliminary observations on cats (under nembutal anesthesia) indicated that following injury to the lung one could observe alongside the fall in the amplitude of the impulse also a prolongation of discharge on inspiration – up to 2 seconds instead of 1.3 seconds prior to the injury.

Thus, our experiments indicated that at the basis of the phenomenon of "lagging" of respiratory movements on the side of lung lesions are reflex suppression of diaphragmatic activity and simultaneous increase in activity of the contralateral diaphragm.

In connection with this, it must be pointed out that although ontogenetically the diaphragm arises as a paired organ, later on the muscle as well as its innervation unite anatomically as well as functionally to such a degree that some investigators think it possible to regard it as a single unpaired organ [7]. At the same time under pathological conditions when a lesion is present in one of the leaves of the diaphragm, the latter is able like a paired organ to alter its function unilaterally. Thus, compensatory hyperfunction of one of the leaves is well known in the presence of paralysis or paresis of the contralateral phrenic nerve, when the latter is divided or injected with alcohol, and when the muscle tone of the contralateral leaf is diminished following an inflam-

matory process, pneumonectomy, lobectomy, etc. Such compensatory hyperfunction is possible in the presence of severe disturbance of innervation of the diaphragm because of the so-called cross phenomenon of the diaphragm[3].

However, if in the presence of all these pathological processes diaphragmatic hyperfunction could be ascribed to compensation for the injured organ, our experiments indicated that this compensatory exaggeration can be a component of a general reflex reaction which expresses itself in the form of a simultaneous decrease on the side of injury and an exaggeration of contractions on the opposite side. Thus, injury to the lung parenchyma causes reflexly an active dynamic reconstruction of the centers regulating diaphragmatic movements.

At what levels is the force of contraction of the diaphragm determined?

It could be supposed that this depends on the inequality of the strength of impulses from the right and left halves of the respiratory center. Such an assumption is contradicted by the observation that an increase in activity is observed not only in the contralateral diaphragmatic leaf, but also in the respiratory muscles of this same side. Activity of nasal muscles is also increased (see Fig. 2). Apparently the general intensification of the impulse in the respiratory muscles caused by stimulation of the respiratory center when one of the lungs is injured, is actively suppressed only in structures regulating diaphragmatic movement. It can be supposed that for the diaphragm this level is in the 5th and 6th segments of the cervical spinal cord which contain the phrenic nerve center.

Our previous investigations indicated the possibility of such a selective suppression of the diaphragm in the presence of a generalized increase of activity of all other respiratory muscles under conditions of simulated asphyxia [1].

The afferent paths of the reflex "lagging" of the diaphragm on the side of a pulmonary lesion require further study. It is possible to suppose that they must include sensory fibers of the phrenic nerve, inasmuch as there is an abrupt change in the tone of the diaphragm at the moment of lung injury.

SUMMARY

In the presence of a unilateral focal pulmonary lesion the electric activity of the diaphragm is decreased on the homolateral side and increased on the contralateral side. This reflex lies at the basis of the physical sign known as "lagging" of respiratory movements on the side of the pulmonary lesion. The absence of compensatory increase of respiratory movements on the side of the lesion is evidently connected with active suppression of diaphragmatic activity taking place in the phrenic nerve center.

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OXYGEN THERAPY IN THE RECOVERY PERIOD FOLLOWING CLINICAL DEATH

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Management of the recovery period following clinical death is one of the least investigated phases of pathological physiology and therapy in terminal conditions.

In the process of dying, in clinical death and in the recovery period following, one can observe intense hypoxia of various types, as shown by the work of V. A. Negovskii [10], M. S. Gaevskaia [5], E. M. Smirenskaia and E. S. Zolotokrylinaia [16], O. N. Bulanovaia [3]. It is only natural that the matter of abolishing the hypoxia by administration of oxygen presents a fundamental therapeutic problem during the recovery period.

The literature contains a variety of opinions on the subject of administration of oxygen in hypoxia. A number of investigators [4, 19, 23 and others] are of the opinion that administration of 100% oxygen is indicated in hypoxia; the majority of authors [2, 9, 13, 14, 18, 20-22] point out the hypersensitivity of the organism in intense hypoxia to oxygen and the possibility of producing the paradoxical action of oxygen – hyperoxia.

This makes it imperative to investigate the possibility and effectiveness of the use of oxygen in the recovery period following clinical death. The following experiments were carried out in investigating this problem.

EXPERIMENTAL METHODS

We carried out experiments upon 40 adult dogs. A state of clinical death was produced in animals, anesthetized with pantopon and ether, by massive bleeding from the femoral artery. The period of dying lasted from 7 to 20 minutes, clinical death lasted 5 minutes. Vital functions were restored by the method developed by V. A. Negovskii and co-workers. Oxygen therapy was begun at different stages of the recovery period following clinical death and was administered by methods used in clinical medicine: artificial respiration during resuscitation, oxygen inhalation from a bag or in an oxygen tent after restoration of spontaneous respiration. Depending on the method employed the experiments were divided into three groups. In group III determinations of the gaseous content of blood flowing to and from the brain and of the rate of blood flow were made by Komissarenko's method.

The effectiveness of oxygen therapy was determined by estimating the general condition of the animals (pulse, respiration, temperature, blood oxygen saturation, pulmonary ventilation, rate of blood flow, etc.), the survival period following clinical death and the morphological changes. Viability of the animals after clinical death was determined by the time it took to re-establish respiration. According to V. A. Negovskii's data, complete restoration of vital functions takes place in 1-3 days in animals which were denied oxygen therapy and in which respiration was re-established in 1½-2 minutes following clinical death of 5 minutes duration; in animals in which respiration was re-established in 4-5 minutes complete restoration of vital functions occurred in 4-6 days and only in 60% of the cases, and in animals in which re-establishment of respiration took 7-9 minutes the mortality rate in the first day was 98-100%.

Histological examination of the brain and viscera was performed on 20 dogs in group III. Fourteen of the 20 died in 1-5 days, one on the 30th day and 5 were sacrificed on the 14th, 47th, 67th, 75th and 97th day, respectively, following the experiment. The brain was stained by the method of Nissl, Snesarev (for astrocytic glia, argyrophilic granules and connective tissue), Miiagav (as modified by Alexandrovskaia) and Bil'shovski. The viscera was stained for fat with hemotaxylin-eosin according to the method of Van Gieson.

EXPERIMENTAL RESULTS

In the 9 dogs of group I in which artificial respiration using 100% oxygen was administered at the rate of 24-28 per minute, it took 2-2½ times as long to re-establish spontaneous respiration. When apparatus inducing active inspiration and expiration was employed, spontaneous respiration was re-established in 4-5 minutes and in control animals in 1½ -2 minutes; only in one dog was respiration re-established rapidly – in 2 minutes, as in the controls. When apparatus for inducing artificial respiration was used only during active inspiration, respiration was re-established in 5-8 minutes and in the controls 2-4 minutes. The delay in re-establishing respiration in these experiments was due, according to the data in the literature ([24] and others), to central nervous system depression, particularly the respiratory center, by oxygen. The delay was not great because superimposed on the depression of the respiratory center was the sensitivity of the center to reflex stimulation brought about in our experiment by means of artificial respiration. Most of the experimental animals in this group (7 of the 9), including the dog in which there was no delay in re-establishing of respiration, perished. In the remaining 2 animals artificial respiration with oxygen had no effect: in them complete re-establishment of vital functions was observed after an interval typical for controls (see Table).

TABLE

Results of Oxygen Therapy Following Clinical Death from Blood Loss

Experiment group	Type of oxygen therapy	No. of experiments and oxygen dosage	Results			
			animals died in 1-5 days	no effect	prolongation of life	complete re-establishment of respiration
I	Artificial respiration with oxygen	9 expts. 100% oxygen	7 (2-8 min)	2 (3-4 min)	—	—
II	Inhalation of oxygen from a bag	9 expts. 100% oxygen:	3 (3-3½ min)	—	—	—
		3 expts.–5 min. every 20 min.	—	1 (10 min)	4 (5—7 min) 8—47 days	1 (5½ min) 46 hrs
		6 expts.–3 min. every 30 min.				
III	Inhalation of oxygen in tent	22 expts.				
		3 expts.–70% oxygen, 2 hrs	3 (1½—2 min)	—	—	—
		12 expts.–40-50% oxygen, 2 hrs.	—	12 (2—10 min)	—	—
		7 expts.–40-50% oxygen, 4 hrs.	—	—	4 (6½—9½ min) 8—97 days	3 (4½—7 min) 2—3 days
total (40 dogs)			13	15	8	4

Note: The time consumed in re-establishing respiration is shown in parentheses.

In group II (9 dogs) inhalation of 100% oxygen during the recovery period was administered from a rubberized bag using a mask equipped with inspiration and expiration valves. Inhalation was begun 45-60 minutes following the beginning of resuscitation, when, according to E. M. Smirenskaia [15] and M. S. Gaevskaia [5], the brain begins to consume oxygen more actively, changing from glycolysis to oxidation of carbohydrates. Respiration at this time was spontaneous, 22-28 per minute and arterial blood pressure was 100-130 mm of mercury.

The results obtained in these experiments depended upon the dosage of oxygen. In 3 dogs in which respiration was reestablished in 3-3½ minutes, inhalation of oxygen from the bag for 5 minutes at 20 minute intervals quickly caused deterioration of their condition. After 6-9 such sessions clonic and tonic convulsions of the extremities and a rise of the respiratory rate to 45-54 per minute were observed in the previously quiet animals. When oxygen therapy was continued stronger convulsions extending to the musculature of the trunk and head were observed, the respiratory rate rose to 120-170 per minute and profuse bloody stools were expelled. The dogs died after 10-18 sessions of oxygen therapy over a period of 13-19 hours from the time of resuscitation. The clinical picture and morphological findings corresponded to the symptoms of hyperoxia, described by a number of workers [6, 8, 13, 25-27]. In healthy animals such dosage of oxygen caused no change in their condition. From the data available in the literature one may assume that the most likely mechanism of hyperoxia consists in diminution of ability to accommodate to the hypoxia and in the accumulation in the tissues of carbon dioxide which has a narcotic effect.

As the period of oxygen inhalation was lowered to 3 minutes, the interval increased to 30 minutes and the sessions repeated 8-9 times per day over a period of 5-9 days, oxygen therapy in one dog failed to influence the process of recovery and it died on the first day, as anticipated because of the longer period required to reestablish respiration (10 minutes; see Table). In the remaining 5 dogs there was some clinical evidence of the favorable effect of oxygen therapy. It took the form of prolongation of life to 5-47 days in obviously nonviable dogs with delayed re-establishment of respiration (6-7 minutes). However, complete re-establishment of vital functions was observed in only one of these dogs and took place in 46 hours. Under such conditions controls die within the first day.

In group III (22 dogs) inhalation of air enriched with oxygen to 40-70% was administered in an oxygen tent during the recovery period. The results also depended on the dosage of oxygen and the time when oxygen therapy was started. Three viable dogs with periods of re-establishment of respiration of 1½-2 minutes which were given inhalation of 70% oxygen for 2 hours per day died during the first day with symptoms of hyperoxia. In 2 of these, oxygen therapy was begun 45-60 minutes and in one – 90 minutes after the beginning of resuscitation, when the brain already actively utilizes oxygen. In the case of healthy animals placed in an atmosphere of oxygen of similar concentration for a period 2-3 times greater, no alteration of their condition or harmful effects were noted.

However, in the case of one dog not subjected to clinical death, but in a state of neurosis (established by the method of conditional reflexes), a one hour exposure in an oxygen tent containing 75% oxygen resulted in an abrupt motor excitement and dyspnea (120 respirations per minute) with death following 13 minutes after removal from the tent and due to hyperoxia as was confirmed by morphological examinations.

These experiments indicated the necessity of lowering the oxygen content in the tent, and for the following 12 dogs its content was reduced to 40-50%. That such oxygen dosage is more rational is evident from data in the literature [1, 7, 11, 17, 21, 28, etc.]. Exposure of animals to such a content of oxygen in a tent for 2 hours 40-60 minutes from beginning of resuscitation did not influence the process of subsequent recovery and the outcome of the experiment was determined by the period of re-establishment of respiration (see Table). Oxygen saturation of the blood of these animals rose by 2-4% (from 91-96 to 94-98%) only while they remained in the tent and rapidly fell to initial values when removed from the tent.

Consequently, in the next 7 experiments on animals with longer periods of re-establishment of respiration (4½-9½ minutes) we extended administration of oxygen therapy to 4 hours a day using concentrations of 40-50%; the sessions were instituted 80-112 minutes after beginning resuscitation and were repeated during the following 2-15 days. In 3 animals in which respiration was re-established in 5-7 minutes, complete recovery of vital functions took place; in the remaining dogs life was prolonged to 8-97 days, but significant neurological disturbances were observed.

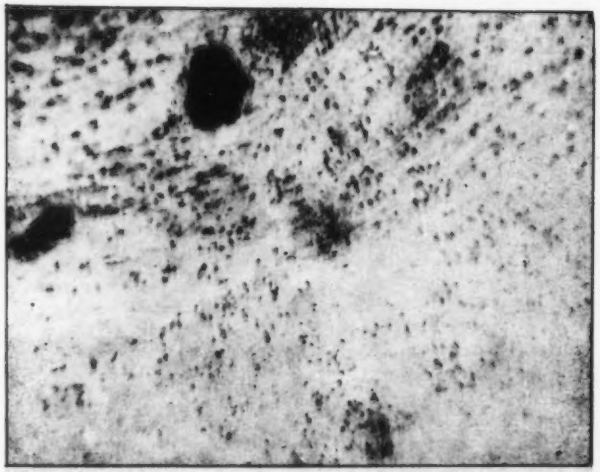

Fig. 1. Numerous small hemorrhages in the cortex of the brain of a dog which endured a state of hypoxia. Stained according to Nissl. Magnification 7 x 8.

Fig. 2. Thickening and homogenicity of the vessel wall in the brain of a dog which endured a state of hyperoxia. Stained according to Nissl. Magnification 7 x 40.

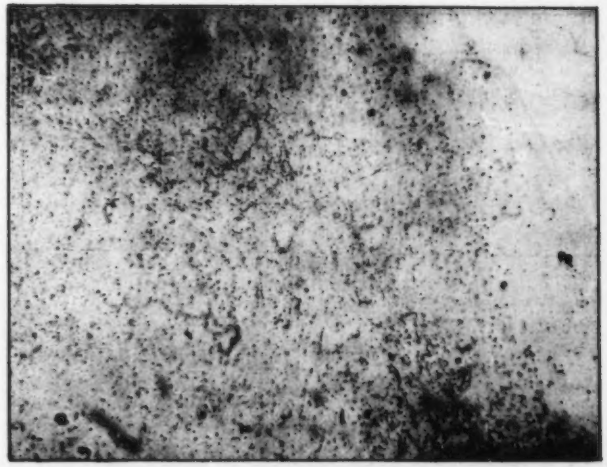

Fig. 3. Extensive focus of necrosis of nerve cells in the cortex of the brain of a dog with delayed re-establishment of respiration, sacrificed on the 67th day after the experiment. Stained according to Nissl. Magnification 7 x 8.

The animals lay quietly in the tent, the pulse rate fell (from 70-150 to 50-90), moderate slowing down of blood flow (1½-2 times) and of pulmonary ventilation were noted. Respiration and body temperature showed no uniform changes. Changes in oxygen saturation of the blood were slight – 2-4% and only while in the tent. The normal difference between oxygen and carbon dioxide levels in arterial and venous blood was observed to increase and a rise in the carbon dioxide level in both arterial and venous blood and in the oxygen content of arterial blood was observed. These changes in the gas content of blood were analogous to the findings obtained by us in experiments without administration of oxygen therapy [15].

Histological examination of the brain disclosed diffuse and focal changes; their character varied depending upon the dosage of oxygen and the length of survival after the experiment. Diffuse changes in the nerve cells, glia and argyrophilic granules did not differ from those observed by us in animals which were not given oxygen therapy as a resuscitation measure after a 5 minute clinical death, i. e. controls [12].

Characteristic of the animals which exhibited the clinical phenomenon of hyperoxia and which perished in the days immediately following the experiment, was the large number of hemorrhages (Fig. 1), perivascular edema and changes in vessel walls (Fig. 2) in all parts of the brain.

The animals with delayed restoration of respiration (5-9½ minutes) which we succeeded in keeping alive 8-97 days were regarded by us from the clinical standpoint as having benefited somewhat from the effect of oxygen. Histological examination revealed considerable changes in these animals. In the cortex, cerebellum and the horn of Ammon were found numerous areas with small foci of necrotic nerve cells; in the cerebellum, besides the death of the cells of Purkinje, there were extensive areas of rarefaction in the granular layer. Such large numbers of widely scattered areas of nerve cell necrosis were never seen in controls. Besides, in some of these animals we discovered focal changes in the cortex with destruction of tissue architecture (Fig. 3). Depending on the length of survival of the animal after the experiment, various stages of scar organization were observed at the site of focal tissue destruction.

In the dog with neurosis, which died after a one hour stay in the oxygen tent, numerous hemorrhages and acute congestion of the viscera were observed at autopsy. Microscopic examination disclosed numerous hemorrhages in the brain and viscera as well as acute swelling of many nerve cells in various parts of the brain.

In the internal organs, besides changes also seen in controls (hyperemia, edema) and depending on the severity of hypoxia suffered during clinical death, were seen numerous hemorrhages and changes in vessel walls similar to those in the brain. Pneumonia with large numbers of epithelial cells within the alveoli was found in almost all the animals.

Investigation indicated that in animals undergoing clinical death there was an alteration is response and consequently administration of oxygen resulted in death of 13 out of 40 during the first day; in 15 animals oxygen therapy had no effect and the outcome of the experiment depended, as in controls, upon the period of re-establishment of respiration; in 8 nonviable dogs oxygen therapy permitted prolongation of life to 5-97 days, but all animals died and in their brains marked changes, including focal ones, were found; only in 4 animals was there evidence of favorable effect of oxygen therapy and in these complete restoration of vital functions was observed in association with longer periods of re-establishment of respiration (4½-7 minutes). Therefore animals undergoing clinical death possess an increased sensitivity to oxygen. Its administration easily produces hyperoxia and leads to death.

Artificial inhalation of 100% oxygen in resuscitation from clinical death appears to be inexpedient. The dosage of oxygen must be strictly supervised when oxygen therapy is given during the recovery period. A limited favorable effect of oxygen therapy is produced when oxygen therapy is administered in a tent containing 40-50% oxygen for not less than 4 hours and starting 80-112 minutes after beginning of resuscitation.

The elimination of hypoxia during the recovery period is not a basic therapeutic measure. Strictly controlled oxygen therapy, it seems, is more effective when combined with other therapeutic measures.

SUMMARY

Forty experiments were carried out on dogs with the employment of oxygen therapy during the period of recovery from clinical death caused by acute hemorrhage. Oxygen therapy was carried out by 3 methods commonly used in the clinic: by an apparatus for artificial circulation, by inhalation of oxygen from a pillow or

in an oxygen tent after rehabilitation of spontaneous respiration. The efficacy of oxygen therapy was judged by the general condition of animals, the span of life after the clinical death and by the morphological changes in the brain and internal organs.

The animals which are in condition of hypoxia after clinical death possess an increased sensitivity to oxygen and hyperoxia may easily occur, causing their death. Artificial respiration with 100% oxygen in resuscitation during clinical death is not expedient. During the recovery period after clinical death strict dosimetry of oxygen therapy should be observed. Certain favorable effects are obtained only when resuscitation is carried out for 4 hours in a tent with oxygen content of 40-50% in 80-112 minutes after commencing the resuscitation.

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STUDY OF THE SORPTIVE PROPERTIES OF SOME ORGANS IN EXPERIMENTAL TUBERCULOSIS, USING THE SUPRAVITAL STAINING METHOD

COMMUNICATION III. SORPTIVE PROPERTIES OF THE SPLEEN AND LUNGS

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(Received July 27, 1957. Presented by Active Member AMN SSSR D. N. Nasonov (deceased))

We have applied the supravital staining method to the evaluation of the degree of injury suffered by tissue elements at different stages of development of experimental tuberculosis. Parts I and II of this series dealt with changes in the sorptive properties of the cerebral cortex and skeletal muscles of guinea pigs. Our observations confirmed the possibility of applying this method to the detection of early, premorphological changes taking place in the tissue elements of the brain and the muscles of infected animals, as well as to the quantitative comparative assessment of the degree of injury suffered by these organs at different stages of the tuberculous process, and to the study of the specific features of the development of the lesions of the tissue elements in time. As is known, the organs investigated by us are not greatly affected by the specific tuberculous process. It was therefore necessary to extend our investigations to such tissues as the spleen and the lungs, which suffer severe injury as a result of the specific inflammatory lesions of tuberculosis.

EXPERIMENTAL METHODS

We investigated the sorptive properties of the lungs and spleen of guinea pigs, the brain and muscles of which had been studied in our earlier researches.

For determination of the sorptive properties of the lungs, we opened up the thoracic cavity, removed the contents, and placed them on a muslin napkin moistened with Ringer solution. We removed the heart, the pleura, and the esophageal stump. We ligated the trachea, and at the same time attached a weight (a glass sphere) to the preparation, which was then washed free of blood in a stream of Ringer solution. The spleen was similarly freed of surrounding tissues, and washed in Ringer solution. The organs were then immersed for 30 minutes in 0.01% neutral red solution at 20-22°. The stained organs were then rinsed in Ringer solution, any remaining pleural tissue was removed, as well as the lymph glands of the root of the lungs, and the spleen was suspended in a test tube, and the lungs in a large cuvette, containing a fixed volume of acidified 70% alcohol (2% solution of sulfuric acid), for extraction of the dye. Extraction of the sorbed dye was complete within 24 hours.

The determination of the amount of sorbed dye, the weighing of the dry organs, and the statistical treatment of the results of each experiment and of the control series, were done according to the same general procedures for all the experiments (Part I).

We performed 800 determinations in 3 series of experiments (400 for each organ). In the first series the animals were inoculated with a virulent culture of M. tuberculosis, bovine type, in the second series with an attenuated BCG culture, and in the third series with the virulent culture, following previous immunization with BCG culture (two vaccinations).

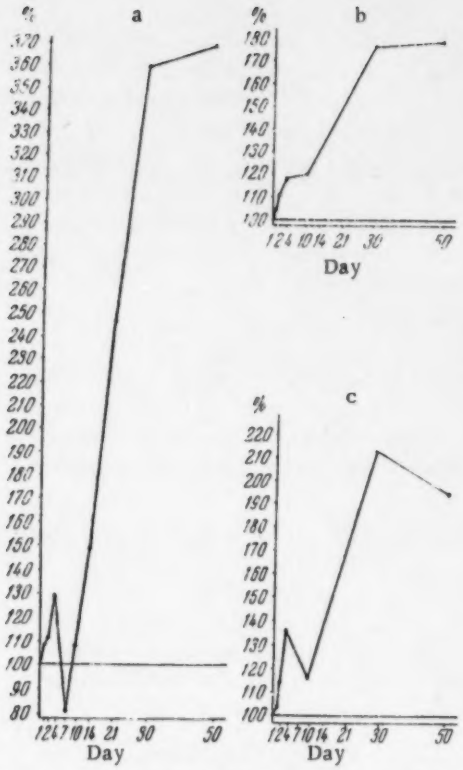

Fig. 1. Curves expressing change in dry weight of guinea pig spleens during the course of experimental tuberculosis.
Ordinates: dry weight of the spleen, taking 100% as the weight found in healthy control animals; a) inoculation of the animals with a virulent bovine type culture of M. tuberculosis, b) inoculation with an attenuated bovine type culture, c) inoculation of previously immunized animals (two vaccinations) with virulent bovine type culture.

The lungs and spleen were examined 1, 2, 4, 7, 10, 14, 21, 30 and 50 days after infection with virulent culture, and 1, 2, 4, 10, 30 and 50 days after infection with attenuated BCG culture, and after infection with virulent culture following BCG vaccination.

Pathological examinations of the viscera of a group of animals, specially set aside for this purpose, were made at the same time intervals, and parallel with the above experiments.

EXPERIMENTAL RESULTS

Solitary tubercles appeared in the lungs 10 days after inoculating guinea pigs with virulent mycobacteria; they consisted mostly of epithelial cells. On the 50th day of infection the lungs of all the animals contained multiple, confluent tubercles, made up of epithelioid cells. Necrosis was evident at the center of many of the tubercles. Solitary epithelioid tubercles made their appearance 30 days after inoculation of virulent culture into previously immunized animals. The number of tubercles was somewhat greater towards the 50th day of infection. At no time were any necrotic changes found in the tubercles of the animals of this series.

Solitary epithelioid tubercles were found in the spleen of guinea pigs inoculated with virulent culture, from the 7th day of infection. By the 30th day the tubercles were multiple and confluent. Solitary epithelioid cell tubercles made their appearance between the 10th and the 30th day of inoculation of previously immunized guinea pigs with virulent culture. Multiple, confluent tubercles were present on the 50th day.

In order to evaluate the results of our study of sorptive properties of the lungs and spleen of the experimental animals we had first to ascertain how the dry weight of these organs varied, bearing in mind the considerable intensification of inflammatory processes with them, as the disease progressed.

Figure 1 shows the changes in dry weight of the spleen at various times (abscissae) after inoculation of the various groups of animals. A steep rise is found in the dry weight of the lungs, and particularly of the spleen, of animals inoculated with virulent cultures, and this rise is most pronounced in the advanced stages of the disease. A considerable rise in the dry weight of the spleen is also evident in the two other series of experiments, being more pronounced when the previously immunized animals were inoculated with virulent culture; the differences in the weight of the lungs are much smaller in these series.

Our results show that changes in dry weight of the organs must be taken into account in evaluating the changes in sorptive properties at certain definite stages of development of the morbid process. For infection with virulent cultures of M. tuberculosis this stage is reached on the 14th day of infection, in the spleen, and on the 21st day for the lungs. Inoculation of nonimmunized animals with BCG culture, and of immunized animals with virulent culture, is followed by change in the dry weight of the spleen from the 30th day on, and by change in the dry weight of the lungs at the same time, but only in animals infected with virulent culture.

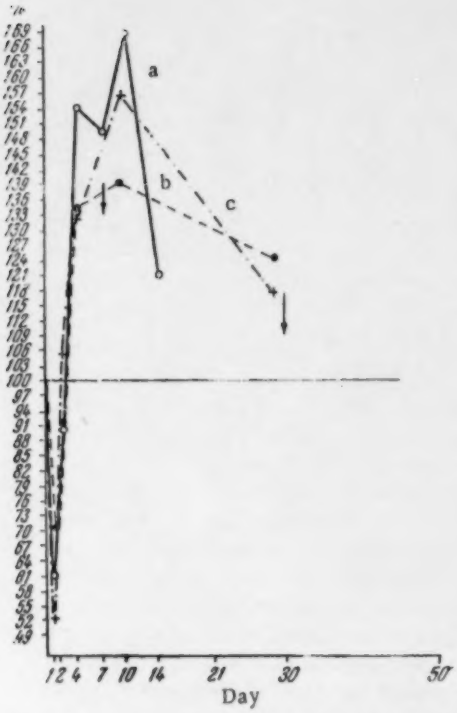

Fig. 2. Changes in sorption of neutral red by the spleen of guinea pigs suffering from experimental tuberculosis.
Ordinates: amount of sorbed neutral red, taking as 100% the amount taken up by the spleen of control (healthy) animals; a) after inoculation with virulent bovine type M. tuberculosis culture, b) after inoculation with attenuated BCG culture, c) after inoculation with virulent culture of previously twice immunized animals. The arrows indicate day of appearance of tubercles.

Results of Supravital Staining Experiments

The results of the experiments on sorption of neutral red by the spleen and lungs of the three groups of animals at different times after infection (abscissae axis) are illustrated in Figs. 2 and 3.

Tables 1 and 2 show the differences between the amounts of dye bound by the spleen and lungs of the experimental and the control animals, at different times after infection, and also the mean square error of the numerical results.

Comparison of the sorptive properties of the lungs and spleen shows that in all three series of experiments the sorptive capacity of these tissues undergoes a series of fluctuations during the course of the infection, the amplitude of the fluctuations being greater with virulent than with attenuated cultures.

The sorptive capacity of the lungs and spleen of the experimental animals varies biphasically. The first phase is distinguished by a fall in sorptive capacity, taking place within a few hours of infection. The duration of this phase is the same in all three series of experiments, but its magnitude varies according to the experimental conditions.

The second phase is characterized by a rise in sorptive capacity, to a different extent for each series of experiments. Transition to the second phase takes place with an abrupt rise in the curves (Figs. 2 and 3), beginning with the 2nd day after inoculation. The curves reach a first peak on the 4th day after inoculation. A second peak in the curves for the spleen and lungs is seen on the 10th day after inoculation with virulent mycobacteria; when the animals are inoculated with BCG culture the curve falls for the lungs as from the 4th day, but rises for the spleen.

The curves continue to rise, for both lungs and spleen, after the 4th day following inoculation of previously immunized animals with virulent culture, after which they both fall smoothly.

The sorption curves for the spleen and lungs are of the same shape as those found previously for other organs (brain, muscles), with the same biphasic course of the process, characteristic of all organs during the progress of the tuberculous infection; quantitative differences in the reaction of each organ are, however, present.

Comparison with the results of our earlier work shows that the sorptive capacity of the lungs and spleen is not only higher than that of the animals of the control series, but that it also exceeds that of the brain and muscles, under comparable experimental conditions. The greatest differences between the sorption curves are found in the organs of animals inoculated with virulent culture.

The results of histopathological examination of the lungs and spleen, considered in conjunction with their sorptive properties, showed that whereas microscopic evidence of specific inflammatory processes could be perceived in the spleen only from the 7th day, and in the lungs from the 10th day, after inoculation, the supravital staining method revealed profound changes in the protoplasmic substance of the cells of these organs from the first day of the infection. The supravital staining method thus permits of the detection of changes in the cell

TABLE 1

Binding of Neutral Red by the Spleen of Healthy Guinea Pigs and of Guinea Pigs Infected with Tuberculosis Germs, Expressed in Terms of Percentages, and the Mean Square Error.

Infected with	No. of expts.	Number of days after inoculation								
		1st day	2nd day	4th day	7th day	10th day	14th day	21st day	30th day	50th day
Virulent M. tuberculosis	10—30	—40±8.32	—9.8±2.26	+54.0±6.8	+49.2±11.1	+68.8±11.5	+25.8±4.55	+63.6±10	+44.2±11.7	+19.0±4.16
BCG	3	—29.3±1.85	—8.7±2.35	+34.7±4.1	—	+39.0±6.9	—	—	+24.7±3.7	+3.3±1.2
BCG + virulent M. tuberculosis	3	—48.3±2.01	+5.3±13.56	+32.0±8.06	—	+56.7±6.88	—	—	+18.3±2.9	+36.7±11.0

Note: the figures preceded by the signs + or - signify increase or decrease in the amount of dye bound by the spleen of the inoculated animals, as compared with the amount bound by the spleen of healthy controls, taken as 100%. Figures preceded by the sign ± are the mean square errors. The values given in the columns represent the arithmetic means of the total number of determinations for the given day.

TABLE 2

Binding of Neutral Red by the Lungs of Healthy Guinea Pigs and of Guinea Pigs Infected with Tuberculosis Germs, Expressed in Terms of Percentages, and the Mean Square Error.

Infeeted with	No. of expts.	Number of days after inoculation								
		1st day	2nd day	4th day	7th day	10th day	14th day	21st day	30th day	50th day
Virulent M. tuberculosis	10—30	—24.3±1.45	—15.0±2.09	+67.2±19.2	+49.0±28.6	+72.8±10.4	+15.3±3.2	+15.6±2.6	+19.6±7.8	+14.2±4.5
BCG	3	—21.3±6.9	—24.7±6.38	+33.3±5.1	—	+18.0±3.0	—	—	+8.0±5.67	+10.3±3.17
BCG + virulent M. tuberculosis	3	—36.3±3.84	—30.3±4.66	+49.3±11.05	—	+56.7±16.0	—	—	+26.7±12.7	+10.0±5.0

Note: the figures preceded by the signs + or - signify increase or decrease in the amount of dye bound by the lungs of the inoculated animals, as compared with the amount bound by the lungs of healthy animals, taken as 100%. Figures preceded by the sign ± are the mean square errors. The values given in the columns represent the arithmetic means of the total number of determinations for the given day.

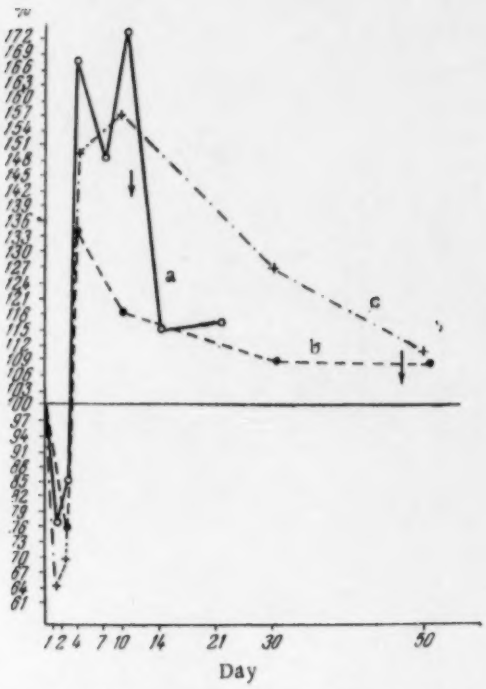

Fig. 3. Changes in sorption of neutral red by the lungs of guinea pigs suffering from experimental tuberculosis.

Ordinates: amount of sorbed neutral red, taking as 100% the amount taken up by the lungs of control (healthy) animals; a) after inoculation with virulent bovine type M. tuberculosis culture, b) after inoculation with attenuated BCG culture, c) after inoculation with virulent culture of previously twice immunized animals. The arrows indicate day of appearance of tubercles.

protoplasm of the lungs and spleen much earlier than the appearance of histopathological changes. It hence follows that the changes in the sorptive properties of the protoplasm, which reflect the physiological state of the cells are among the very earliest and most delicate indications of the reaction of the protoplasm to the very minute changes in the environmental conditions of the cells, due to the tuberculous infection. These changes in sorptive capacity also enable one to make a comparative quantitative evaluation of the changes occurring in the functional state of tissue elements. The appearance of tubercles coincidently with the peak of the sorption curves of both the organs examined points to the interdependence of these phenomena.

SUMMARY

Inoculation of guinea pigs with virulent or attenuated cultures of M. tuberculosis is followed by an immediate fall in the sorptive capacity for neutral red of the spleen and the lungs, to a minimum at the end of the first day, followed by a steep rise to a peak value, which coincides in time with the appearance of tubercles in histopathological preparations. The sorptive capacity fluctuations vary in amplitude parallel with the severity of the tuberculous process, in the ascending order: BCG inoculation, inoculation of immunized animals with virulent culture, inoculation of nonimmunized animals with virulent culture. The changes in sorptive capacity are a far more delicate indication of the very earliest stages of tuberculous infection than are histopathological methods.

BIOCHEMISTRY AND BIOPHYSICS

CERTAIN SPECIFIC FEATURES OF THE HIGHER NERVOUS ACTIVITY OF FULLY GROWN ANIMALS IRRADIATED ANTENATALLY WITH IONIZING RADIATION

COMMUNICATION I. THE INFLUENCE OF IONIZING RADIATION ON THE OFFSPRING

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Received January 17, 1958. Presented by Active Member of the AMN SSSR V. V. Parin)

Irradiation of pregnant female animals and women with ionizing radiation may cause the appearance of a variety of congenital deformities in the offspring and may interfere with their postnatal development [2-6].

L. Hicks [4] points out the particular sensitivity of the nervous system of the embryo to ionizing radiation. Thus irradiation of rats on the 9th, 11th, 12th and 13th days of prenatal development may cause, in addition to somatic deformities, anencephaly (on the 9th day), hydrocephaly (on the 11th day), microcephaly (on the 12th-13th day) — failure of development of the subcortical structures, the corpora callosa and so on.

The influence of ionizing radiation on the nervous system during antenatal irradiation has been studied mainly morphologically [1, 4]. There are no indications in the literature of the state of the higher nervous activity of fully grown animals exposed at various periods of their antenatal development to the action of ionizing radiation.

This impelled us to investigate the effect of ionizing radiation, applied in various doses and at different stages of embryonic development, on the state of the higher nervous activity of animals.

EXPERIMENTAL METHOD

The day of semination was determined in female white rats by the presence of sperm in a vaginal smear. On the 12th day of pregnancy the animals were subjected to a single total irradiation with γ-rays from radioactive cobalt (dose strength — 200 r, dosage rate — 8.3 r/min.). The present report is based on observations made on 52 rats: 30 irradiated (experimental) and 22 controls, born at the same period, but not exposed to the action of ionizing radiation in utero.

Among the group of irradiated rats, in one marked hydrocephalus was observed, in four — anophthalmia, in one — bilateral microphthalmia and in four — unilateral microphthalmia; in addition one blind animal had a deformity of the tail. The remaining 20 animals showed no external, visible congenital deformities. None of the control animals had any visible defects.

Investigations of the higher nervous activity of the experimental and control animals began at the age of 45-50 days (when they had reached a weight of 70-80 g). The experiments were carried out in 2 series, and the motor-alimentary method of L. I. Kotliarevskii was used (the work was undertaken by V. E. Miklashevskii and N. G. Mikhailova).

Initially a conditioned reflex was established to a sound (tone 1), then differentiation to a higher tone was produced (tone 2), after which a conditioned reflex was developed to a light stimulus; a stereotype was then created. In the group of animals with anophthalmia and microphthalmia no reflex to light was established;

both positive reflexes were established to sounds of different frequency. Tone 1 was a sound with a frequency of 400 cps (attenuation 20 db), tone 2 – a sound with a frequency of 800 cps (attenuation 40 db). The light stimulus was the red light from an incandescent lamp (voltage 6v). The duration of the conditioned stimulus was 10 sec: 5 sec in isolation and the next 5 sec in association with unconditioned alimentary reinforcement.

EXPERIMENTAL RESULTS

Observations on the behavior of the animals. During investigation of the conditioned reflexes the normal animal at first usually gives an orientational reflex, which is extinguished after the animal has been placed 4 or 5 times in the chamber. In the experimental animals the orientational reflex is extinguished more slowly. As a rule the experimental animals give a greater number of intersignal reactions. They relatively often show aggressiveness towards each other and towards the experimenter for no cause.

Rate of Appearance and Consolidation of the Conditioned Reflex to Sound

Series of experiments	Experimental animals			Control animals		
	no. of animals	no. of combinations (mean)		no. of animals	no. of combinations (mean)	
		for the appearance	for the consolidation of the conditioned reflex		for the appearance	for the consolidation of the conditioned reflex
1st (expts. by V.E. Miklashevskii)	18	4.5	42	13	5.5	13.5
2nd (expts. by N.G. Mikhailova)	12	10.1	52.6	9	9.3	16.3

Rate of formation and consolidation of the conditioned connections. In the experimental animals the first appearance of the conditioned reflex to sound sometimes takes place rather sooner than in the controls*. Consolidation of the conditioned reflex in the experimental animals always takes place more slowly and requires a larger number of combinations (see Table).

Consolidation of the artificial reflex to light and to the second sound signal also required a greater number of combinations in the experimental group of animals than in the control group.

A difference between the experimental and control groups is also observed when comparing data on the establishment of differentiation, its appearance and consolidation. In the 1st series of experiments the time of appearance of the first negative conditioned reflexes in the experimental and control groups was only very slightly different, but complete differentiation was not achieved in practice in all cases. Of 18 animals in the experimental group consolidation arose in only 12, the number of combinations required being about 20; in the control group, of 13 animals consolidation appeared in 12, the number of combinations being about 12. In the second series of experiments, in the control group of 9 animals complete differentiation was obtained in 8, and on the average 24.8 combinations were required, whereas in the experimental group of 12 animals it was not possible to obtain complete differentiation in all, in spite of a large number of combinations.

Observations on the stereotype. Comparison of the latent periods of the reaction to the positive signals, the magnitudes of the conditioned reflexes and the percentage of loss shows that antenatal irradiation of rats causes a characteristic deterioration in their conditioned reflex activity. On the average the latent period to sound in the experimental animals is equal to 2.65 seconds, and that of the control animals 2 seconds; the magnitude of the reflex is 21.5 and 32 cm respectively; the corresponding percentage loss – 21.5 and 2.5.

*The phenomenon noted may depend on the longer preservation of the orientational reaction in the experimental animals, masking the true time of appearance of the conditioned reflex.

Depression of the conditioned reactions in the second half of the stereotype was observed in a much higher percentage of cases in the experimental rats than in the controls. In 20 experiments the successive inhibition in a group of experimental animals amounted to 40%, and in the control rats – 18%.

Functional tests. In order to ascertain the strength, the mobility and the equilibrium of the nervous processes tests were carried out with an external inhibitor, with prolongation of the differential stimulus to 120-180 seconds, a test of extinction and restoration, and positive induction. In the majority of rats irradiated before birth, on the introduction into the stereotype of an excessively strong stimulus (external inhibitor) a deterioration of conditioned reflex activity was observed, the percentage loss of the conditioned reflexes to sound and light was increased and their magnitude was reduced, and the latent period increased. Tests in which the differentiation was prolonged showed that in the experimental rats in 100% of cases differentiation was disinhibited, whereas the control animals maintained differentiation in 30% of cases.

The study of extinction and restoration of the conditioned reflex showed that the animals irradiated before birth could be distinguished into two groups by the character of extinction of the reflex. In some experimental animals extinction was observed to be more rapid than in the controls. Thus in the control animals complete extinction of the reflex (00000) was reached after 20 stimulations without combination, and in the experimental animals – after 11 (V. E. Miklashevskii). On the other hand in some of the experimental animals extinction took place very slowly. Whereas in the experimental and control groups the beginning of extinction appeared with approximately the same number of combinations, complete extinction was not observed after 70-80 stimulations without reinforcement. The extinguished conditioned reflex was restored slightly more slowly in the experimental than in the normal animals, in the control group – after 2-3 combinations, but in the experimental group – after 2-3-4 combinations.

In the control rats, when the time of action of the positive stimulus was directly after the differentiation, as a rule positive induction was observed, but in the experimental animals this did not happen in the great majority of cases.

The main distinctive feature of the higher nervous activity of rats irradiated before birth, compared with normal animals, is a congenital reduction in the working capacity of their cortical cells. In the rats irradiated before birth the two main nervous processes are less powerful, less mobile and not so well balanced with respect to each other.

In rats irradiated before birth by γ-rays from radioactive cobalt changes were found in the process of internal inhibition, especially extinctive and differential. It can be seen from a number of pointers that sometimes weakening of the process of stimulation did not allow the detection of any disturbance of the process of internal inhibition which might have been present.

The experimental animals also showed a disturbance of the equilibrium of the nervous processes: processes of stimulation were considerably in excess of inhibition. This was shown by the great motor excitation, by the slow attenuation of the orientational reaction and by the large number of intersignal reactions. The mobility of the nervous processes was also disturbed; they showed inertia, reflected in phenomena of successive inhibition.

It may be considered that rats, irradiated before birth by ionizing γ-rays from radioactive cobalt, belong, from the point of view of their type of higher nervous activity, to a weak type in the majority of cases, verging on the pathological – neurosis. It should also be mentioned that as the animals develop and in the course of the work with them, the difference in the indices of conditioned reflex activity between those irradiated before birth and controls becomes smoothed out and less conspicuous.

Morphological examinations of the brain of normal animals and rats irradiated before birth, carried out on the 2nd, 7th, 14th, 30th, 180th and 450th days after birth (by N. I. Artiukhina) revealed a number of characteristic features. In the first seven days of life no difference could be observed in the thickness of the cortex of the experimental and control animals, but in the 14-day-old animals of the control series the cortex was twice as thick as in the experimental animals, in both the frontal and occipital regions. On the 30th and 180th days the thickness of the cortex of the experimental and control animals was the same, but on the 450th day the thickness of the cortex in the frontal region of the experimental animals was again less than in the control animals.

A histological study showed that the nerve cells of the cerebral cortex of the animals irradiated before birth were delayed in formation and maturation. In the control animals the cerebral cortex developed intensively in the period from the 7th to the 14th day of postnatal development, and in those irradiated before birth — in the period from the 14th to the 30th days; in the animals irradiated before birth marked atrophy of the cerebral hemispheres was observed at later periods in consequence of death and atrophy of the nerve cells.

We also observed developmental anomalies as already [4] described in the literature. In 28% of cases the animals irradiated before birth showed developmental defects of the hippocampus, the corpus callosum, the basal ganglia, the optic tubercle, displacement of the rudiments of the subcortical ganglia and hydrocephalus.

SUMMARY

This work was conducted on white rats. On the 12th day of pregnancy the animals were subjected to a total single irradiation (200 r) by γ-rays of radioactive cobalt. The study of the highest nervous activity of rats irradiated during the antenatal period reveals a congenital decrease of the working capacity of their cortical cells as compared to the normal animals. Both fundamental nervous functions are not as strong in the animals irradiated antenatally. These processes are less mobile and less interbalanced. The processes of internal inhibition, especially of extinction and differentiation, are particularly affected.

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CHANGES IN THE ADRENALIN CONTENT OF THE BLOOD AND OF THE AQUEOUS HUMOR OF THE EYE OF THE RABBIT AFTER IRRADIATION

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The reaction of the endoctine glands to the action of ionizing radiation plays an essential role in the development of radiation sickness. Several features, beginning with changes in the blood picture and ending in trophic disturbances of complex origin, are explained by the action of ionizing radiation on the glands of internal secretion. Accordingly it is profitable to study these endocrine mechanisms [2, 3]. According to the ideas of modern radiobiology, the participation of the glands of internal secretion in these reactions is determined by nervous influences on the glands and also by interaction between them. Great importance in these interactions is attached to the reaction of the medullary substance of the adrenals [4]. Some time ago the opinion was expressed that under the influence of ionizing radiation the secretion of adrenalin is intensified [8]. On the other hand great importance must be attached to the increased adrenalin content of the blood as a factor stimulating the reticular substance and exerting an action on all divisions of the central nervous system, including the vegetative centers of the brain stem [5]. It is for this reason that in studying the reaction of the endocrine glands on the action of ionizing radiation, investigation of the adrenalin content of the blood is of particular interest.

EXPERIMENTAL METHOD

The experiments were carried out on male rabbits weighing from 2.5 to 3.5 kg. As an index of the changes in the functional state of the medullary layer of the adrenals we made use of changes in the content of its hormone in the aqueous humor from the anterior chamber of the eye and in the blood. The eye is a convenient object for investigation since chemically active substances enter the aqueous humor via the ciliary processes of the uveal tract. Aqueous humor can be obtained by puncture of the anterior chamber of the eye. Aqueous humor was taken from one eye after 3-5 day intervals in order to avoid damage to the cornea. Before puncture the eye was moistened with a 2% tetracaine solution, and after puncture with a 30% solution of albucid. For quantitative estimation of the adrenalin content a polarographic method was used, as suggested by Henderson and Freedberg [6] for the determination of crystalline adrenaline and modified by us as described in detail in a previous communication.

The animals were irradiated with x-rays in a dose of 800 r under the following conditions: current strength 20 ma, tension 180 kv, focal distance 60 cm, dosage rate from 30.8-33.5 r/min, filter 0.5 mm Cu and 1 mm Al.

Experiments were carried out on the animals at 1-2 minutes, 1 hour and 6 hours after irradiation, and daily until the death of the animal. Altogether 30 rabbits were used in the work. In the rabbits which acted as controls, samples for testing for adrenalin were taken over a period of 10-15 days before irradiation. These samples were the controls. Variations in the adrenalin content in the same animal were insignificant, within limits of 0.01 to 0.035 γ per 1 ml of blood, and from 1.1 to 0.5 γ per 1 ml of blood in the aqueous humor.

EXPERIMENTAL RESULTS

In this paper we took the mean values of the quantitative estimations of the content of adrenalin in the same rabbit obtained in 10-15 experiments. It was found that the mean value of the adrenalin content per 1 ml of aqueous humor was 0.82 γ, and per 1 ml of blood – 0.020 γ.

The numerical findings of the adrenalin content of the aqueous humor are given in Table 1.

After irradiation phasic changes were observed in the adrenalin content (Fig. 1).

From 1-2 minutes after irradiation a clearly expressed diminution in the adrenalin content of the aqueous humor was observed, and in the blood also (to 0.001 γ in 1 ml of aqueous humor and to 0.001 γ in 1 ml of blood, or even lower). In the course of 5-10 minutes the adrenalin content increased slightly – to 1 γ per 1 ml of aqueous humor and to 0.2 γ per 1 ml of blood. In the course of the first hour after irradiation the adrenalin content of the aqueous humor and the blood was observed to be maximum, reaching 15-20 γ per 1 ml of aqueous humor and 25-30 γ per 1 ml of blood. At the end of the first 24 hours the adrenalin content fell almost to the normal value (0.5-0.75 γ per 1 ml of aqueous humor and 0.01-0.025 γ per 1 ml of blood).

TABLE 1

Adrenalin Content of the Aqueous Humor and the Blood of Rabbits

Rabbit No.	Adrenalin content in γ	
	in 1 ml of blood	in 1 ml of aqueous humor
1	0.010	0.5
2	0.015	0.8
3	0.025	1.1
4	0.020	1.2
5	0.015	0.6
6	0.035	0.7

The second wave of increase in the adrenalin content was observed at later stages, preceding the death of the animal (to 20-25 γ per 1 ml of aqueous humor and to 5-5.5 γ per 1 ml of blood) [1, 2].

The numerical findings of the changes in the adrenalin content in the aqueous humor and in the blood during total irradiation are shown in Table 2.

By way of illustration we quote the results of experiments on rabbit No. 4.

The rabbit was kept under observation for 10 days before irradiation, during which time samples of blood and aqueous humor were taken 4 times for adrenalin estimation.

On May 31, 0.4 ml of aqueous humor was taken from the right eye, and the adrenalin content per 1 ml was found to be 1.275 γ, and in the blood – 0.05 γ per 1 ml.

On June 3, 0.3 ml of aqueous humor was taken from the right eye, and 1 ml was found to contain 1.05 γ of adrenalin – and 1 ml of blood 0.0375 γ.

Blood could not be taken from the marginal vein of the ear because of very strong spasm of the vessels.

On June 6, 0.3 ml of aqueous humor was taken from the right eye, and 1 ml was found to contain 0.7 γ, and 1 ml of blood – 0.015 γ of adrenalin.

On June 9, 1 ml of aqueous humor was found to contain 0.8 γ of adrenalin, and 1 ml of blood – 0.02 γ.

On the basis of the results obtained the mean value of the adrenalin content of 1 ml of aqueous humor and of blood was estimated. For the blood this figure was 0.0306 γ, and for the aqueous humor – 0.958 γ. These slightly raised values (in relation to normal) can be accounted for by seasonal variations in the adrenalin content, which reaches a maximum level in June-July [7].

On June 12, the rabbit was given total irradiation with x-rays in a dose of 800 r under the following conditions: current strength 20 mA, tension 800 kv, focal distance 60 cm, dosage rate 33.5 r/min, filter 0.5 mm Cu and 1 mm Al.

From 4-5 minutes after irradiation, 1 ml of blood was found to contain less than 0.01 γ of adrenalin (or 50% of normal); at first it was impossible to puncture the eye since the rabbit was very restless. After 10 minutes the eye could be punctured, and 1 ml of aqueous humor was found to contain 12.3 γ of adrenalin, which amounts to 1280% of the normal value. A second puncture was carried out one hour after irradiation and 1 ml of aqueous humor was found to contain 42.83 γ of adrenalin (or 4471%).

On the first day, the adrenalin content of 1 ml of blood was 0.02 γ, i. e. within normal limits, but on the second day this had decreased to less than 0.02 γ of adrenalin, i. e. slightly below the normal level. On the 3rd day 1 ml of blood contained 0.08 γ of adrenalin and 1 ml of aqueous humor – 0.8 γ. On the 7th day after irradiation 1 ml of aqueous humor contained 7.65 γ of adrenalin, and 1 ml of blood – 0.15 γ (or 798% and 5000%).

TABLE 2

Changes in the Adrenalin Content of the Aqueous Humor and the Blood of Rabbits during Total Irradiation

Rabbit no.	Days after irradiation	Adrenalin content in the aqueous humor of the eye		Adrenalin content in the blood	
		γ	%	γ	%
1	Normal	1.08	100	0.05	—
		—	—	0.0375	—
		—	—	0.0675	—
		—	—	0.02	
				Mean 0.038	100
	10 min.	61.725	5715.2	21.65	57 000
2	Normal	1.0	—	0.05	—
		0.5	—	0.02	—
		0.8	—	0.02	—
		Mean 0,75	100	Mean 0.03	100
	4—5 min	0.01	1.334	—	—
	1-e days	20.00	2666	0.02	66.6
	2-e »	—	—	20.001	66 666.6
	3-и »	—	—	0.08	266.6
	5-e »	0.20	26.6	0.002	6.6
	7-e »	10.00	1 334	4.75	15 833
	Died on the 8th day				

Rabbit no.	Time after irradiation	Adrenalin content in the aqueous humor of the eye		Adrenalin content in the blood	
		γ	%	γ	%
4	Normal	0.8	—	0.02	—
		0.5	—	0.02	—
		Mean 0.65	100	Mean 0.02	100
	10 min	12.45	1 917	—	—
	6 hrs	30.15	4 638	—	—
	3- days	0.65	100	—	—
	5-e »	0.70	108	—	—
	9-e »	40.65	6 269	Died on the 9th day	
6	Normal	—	—	0.01	—
		—	—	0.02	—
		—	—	0.01	—
				Mean 0,13	100
	5 min	—	—	0.001	7.7
	7 hrs	—	—	0.1	100
	3- days	—	—	0.15	100
	5-e »	—	—	0.20	153
	7-e »	—	—	10.2	7 846
	2 hrs before death				

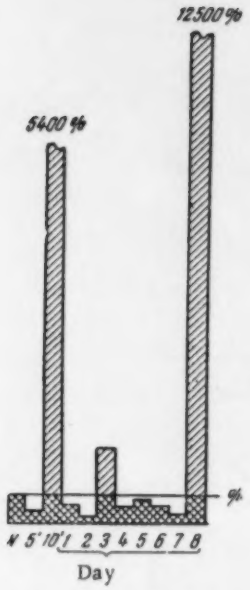

Fig. 1. Changes in the content of adrenalin in the blood of the rabbit (expressed as percentages) after total irradiation with x-rays.

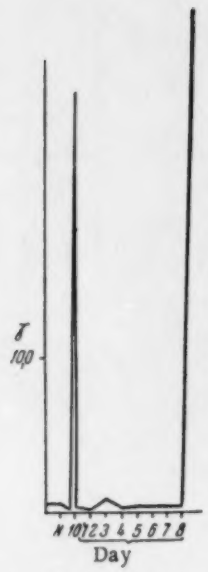

Fig. 2. Changes in the adrenalin content of the aqueous humor of the eye of the rabbit after total irradiation with x-rays.

The rabbit died on the 9th day after irradiation. Two hours before death 1 ml of aqueous humor of the rabbit contained 10.153 γ of adrenalin (or 1059% of normal) and 1 ml of blood – 4.75 γ of adrenalin (or 15,833% of normal).

Fig. 2 shows the variation in the adrenalin content of the aqueous humor of the rabbit after irradiation. From Fig. 2 it can be seen that after total irradiation with a dose of 800 r, clear and regular changes occur in the adrenalin content, which are phasic in character.

The following conclusions may be drawn from these experimental findings. The action of ionizing radiation on the animal is phasic in character: phase 1 – reduction in the adrenalin content of the aqueous humor of the eye and the blood (1st-7th minute after irradiation); phase 2 – increased adrenalin content in the aqueous humor and the blood (from the 8th minute to the end of the first 24 hours); phase 3 – temporary return to normal of the adrenalin content of the aqueous humor and the blood (second day and thereafter); phase 4 – increased content of adrenalin in the aqueous humor and the blood, preceding the death of the animal (from the second day before death and a sharp increase on the day of death).

SUMMARY

The change of the content of adrenalin was studied in the blood as well as in the aqueous humor of the rabbit's eye after total irradiation with the dose of 800 r. The polarographic method was used for the quantitative determination of the adrenalin content. After total irradiation of the rabbit distinct and regular changes of phasic character take place in the content of adrenalin in the blood and in the aqueous humor of the eye.

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PHARMACOLOGY

THE EFFECT OF LARGE DOSES OF PENICILLIN AND STREPTOMYCIN ON THE AMMONIA CONTENT OF BRAIN

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Experimental investigations and clinical observations show that the brain is sensitive to penicillin and streptomycin. The effect of these antibiotics on cerebral tissue leads at times to serious sequelae.

Definite connection between the functional state of the brain in animals and the formation of ammonia in the brain has been shown in a number of studies by G. E. Vladimirov [1], E. A. Vladimirova [2-6] and others. The amount of ammonia in the brain increases when the animals are excited by chemotherapeutic substances or by unconditioned and conditioned reflexes; in the presence of inhibitory states the cerebral ammonia content declines.

With this in mind a study was made of the effect of large doses of penicillin and streptomycin on the ammonia content of the cerebral tissue.

EXPERIMENTAL METHOD

White male rats weighing from 160-200 g were given subcutaneous injections of solutions of highly purified penicillin and streptomycin salts – sodium salt of crystalline benzylpenicillin with activity of 1650 active units/mg and crystalline streptomycin sulfate with activity of 770 active units/mg.

Control animals were given physiologic solution. After 40-60 minutes the animals were frozen in liquid oxygen in toto in a chamber described by E. A. Vladimirova [3]. Following freezing the ammonia content of the cerebral hemispheres was determined using the method of vacuum distillation with steam in a Parnas apparatus with subsequent addition of Nessler's reagent to the distillates and colorimetric examination. In addition to determination of ammonia the concentration of the antibiotic in the brain was estimated biologically in the majority of animals.

EXPERIMENTAL RESULTS

The results of determinations of ammonia in the brain of the animals are presented in Table 1.

The data shown in Table 1 show that penicillin in doses of up to 500,000 active units, streptomycin sulfate in doses of up to 100,000 active units and the calcium chloride complex of streptomycin in doses up to 200,000 units exerted no effect on the ammonia content of rat brain. With very large doses of penicillin (1 million active units per rat) there is an approximately $1\frac{1}{2}$-fold increase in the ammonia content.

Penicillin concentration in cerebral tissue following administration of 100,000 active units of the antibiotic amounted to only 2-4 active units per 1 g tissue. The concentration was 10-15 active units per 1 g tissue following administration of 400,000-500,000 active units of penicillin. When 1-1.5 million active units of penicillin

TABLE 1

Ammonia Content of White Rat Brain 40-60 Minutes after Subcutaneous Administration of Various Doses of Penicillin and Streptomycin.

Norm	Penicillin		Streptomycin		
NH_3, in mg%	Dose of antibiotic, in thousands of active units	NH_3, in mg%	Streptomycin preparation	Dose, in thousands of active units	NH_3, in mg%
0.35	100	0.48	Streptomycin sulfate	50	0.45
0.36	200	0.42	ditto	65	0.37
0.43	200	0.47	» »	100	0.38
0.48	300	0.41	» »	100	0.40
0.51	400	0.44	» »	100	0.41
0.51	500	0.39	» »	100	0.47
0.52	500	0.46	» »	100	0.55
0.54	500	0.57	» »	100	0.57
0.59	—	—	» »	—	—
mean 0.48	—	mean 0.46		—	mean 0.45
—	1 000	0.57	Calcium chloride complex of streptomycin	100	0.41
—	1 000	0.59	ditto	100	0.44
—	1 500	0.64	» »	100	0.56
—	2 000	0.89	» »	200	0.53
—	2 500	0.87	» »	200	0.53
		mean 0.71		—	mean 0.49

were injected the concentration of the latter was 25-35 active units, and when 2-2.5 million active units were given the concentration was 30-45 active units per 1 g tissue. If it is taken into account that part of the penicillin determined in the brain was in the blood it can be taken that penetration of penicillin into cerebral tissue is slight. It is evident that when massive doses of penicillin (over 1 million active units) are given to animals those relatively small amounts of the antibiotic which penetrate into cerebral tissue are able to evoke a state of excitation and lead to an increase in the ammonia content.

The following fact deserves attention. If rats are given the potassium and not the sodium salt of penicillin an increase in ammonia content ($1\frac{1}{2}$-2-fold) is already noted when the administered dose amounts to 500,000 and 1 million active units. Experiments with subcutaneous injection of potassium chloride demonstrated that high concentrations of potassium ions themselves led to increased ammonia content.

Another antibiotic, streptomycin, penetrated into cerebral tissue to a greater extent. Thus, following administration of 100,000 active units of streptomycin sulfate the concentration of the antibiotic in the brain one hour later fluctuated from 62 to 288 active units per 1 g tissue. The behavior of the animals which received penicillin and calcium chloride complex of streptomycin did not differ from the behavior of the control animals.

Administration of large doses of streptomycin sulfate (100,000-150,000 active units) produced after 30-40 minutes a state of profound inhibition in some rats. The animals became immobile and ceased to react not only to the stimuli but even to touch. The ammonia content of the brain was determined in several of these animals after freezing. The results of these experiments, shown in Table 2, indicate an appreciable decrease in the ammonia content of the brain.

TABLE 2

Ammonia Content of White Rat Brain after Subcutaneous Injection of Large Doses of Streptomycin Which Produced a State of Inhibition in the Animals.

Dose of streptomycin sulfate in thousands of active units	NH_3 in mg%
100	0.22
100	0.27
150	0.20
150	0.25
150	0.28
	Mean 24.4

The streptomycin content of the brain in these animals varied from 384 to 600 active units per 1 g tissue. Hence in these experiments relatively high concentrations of streptomycin in the brain led to a state of inhibition which was rapidly reflected in lowering of the ammonia content.

The results obtained show that penicillin and streptomycin can cause changes in the ammonia content of the brain only when their concentration in cerebral tissue reaches relatively high levels; the character of such change is different for penicillin and streptomycin: the former can increase the ammonia content of the brain, which indicates that this antibiotic may be regarded as a central nervous system stimulant whereas streptomycin, which can lower the ammonia content of the brain, produces inhibition of the central nervous system.

It would seem unlikely that the intramuscular injection of the usual therapeutic doses of penicillin and streptomycin would cause disturbances of the functional state of the nervous system and changes in ammonia content of the brain in man, since streptomycin and especially penicillin penetrate into the cerebral tissue slowly. However, in the presence of impairment of the blood-brain barrier and in intrathecal injection of the antibiotics conditions may arise in which the antibiotic concentration in the brain may be high enough to cause disturbances of the functional state of the nervous system and changes in the ammonia content.*

SUMMARY

Experiments on rats demonstrate that large doses of penicillin and streptomycin may cause changes in the content of ammonia in the brain (penicillin increases and streptomycin decreases the content). These changes are considered to be due to the effect of these antibiotics on the central nervous system (a stimulating effect of penicillin and a depressing one of streptomycin).

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** In Russian.

MICROBIOLOGY AND IMMUNITY

THE EFFECT OF THE TITER OF IMMUNE ANTIBODIES ON THE GROWTH OF TUMOR AND NORMAL TISSUES IN VITRO

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There is a relatively large number of experimental investigations devoted to the question of the action of immune sera on tissue growth in vitro. However, the data furnished by these studies are very conflicting. Thus, R. Ludford [11], H. Phelps [14] and others deny specificity of action of antitumor sera in vitro. M. S. Lomakin [2], A. D. Timofeevskii [6], P. P. Filatov [7], K. Fellinger [8], D. Imagava [9], D. T. Imagava, J. T. Syverton, J. J. Bittner [10], T. Lumsden [12], T. Lumsden, T. F. Macrae, E. Skipper [13], K. Takeda [15] and others on the contrary consider that immune sera can suppress the growth of tumor cells in tissue cultures. Unfortunately the majority of the authors cited do not give precise information concerning the titer of the sera used by them.

With the data of our previous work [4], [5] on the inverse relationship of intensity of tissue growth in vitro and the level of the titer of normal nutrient medium antibodies in mind, we undertook to establish in the present work, begun as far back as 1954, the importance of the titer value and specificity of immune antibodies for the growth of tumor and normal tissues in vitro.

EXPERIMENTAL METHOD

White mouse spleen served as a specimen of normal tissue. Tumor tissue was that of the subcutaneous form of Ehrlich's adenocarcinoma. The tumor and normal tissues were explanted in Carrel dishes. Into each dish were placed 4-5 explants measuring approximately 1 mm^3.

The nutrient media used included cock plasma, chick embryo extract, Tyrode solution and rabbit serum, normal or immune, of different titer and specificity: antitumor sera with antibody titer of 1:20 +++, 1:40++(+), 1:640+++, 1:800+++; antispleen (white mouse) sera with antibody content of 1:40+++, 1:400++, 1:800 +++ and 1:800+++(+); antiliver (white mouse) sera with titer of 1:20++, 1:40++, 1:360+++, 1:400+++. The antibody titer values cited here are given only with respect to specific antigens, since these sera reacted with other nonspecific antigens giving a lower titer. The Ehrlich adenocarcinoma explant and that of white mouse spleen were cultured simultaneously in normal and all the immune sera mentioned above.

A total of 8 series of experiments was carried out; the number of explants in separate series varied from 235 to 347. The total number of explants reached 2475. All the dishes containing explants were subjected to microscopic examination on the 2nd, 4th, 6th, 8th, 10th and 14th day from the start of culturing. On the 6th, 10th and 14th day drawings were made (with the help of the drawing apparatus "RA") of the fragments and their zones of growth, and the ratio of the area of growth zone to the area of the tissue fragment was calculated according to the method described in our previous communication [4].

TABLE 1

Intensity of Growth of Ehrlich Adenocarcinoma Tissue on the 10th Day of Culturing

No. of series	Total no. of explants	No. of explants in each series	Ratio of area of growth zone and area of Ehrlich adenocarcinoma explant						
			sera						
			anti-Ehrlich's adenocarcinoma		antispleen (white mouse)		antihepatic (white mouse)		Normal non-immune
			serum titer						
			1:20—1:40	1:640—1:800	1:40	1:800	1:20—1:40	1:360—1:400	
			experimental group						
			I	II	III	IV	V	VI	VII
1	1284	136	6.76	1.57	6.65	2.49	5.92	6.12	5.93
2		173	5.99	1.21	5.73	1.78	6.41	5.82	6.06
3		170	6.83	1.65	6.17	2.01	6.12	6.45	6.25
4		158	6.44	1.40	6.36	2.51	5.78	6.19	5.73
5		172	6.0	1.42	5.77	1.89	6.01	6.07	6.73
6		170	6.96	1.17	6.20	2.37	5.80	5.95	6.21
7		164	6.69	1.74	6.63	2.12	6.22	5.68	5.87
8		141	6.89	1.20	6.69	2.63	5.69	5.98	6.01
		Mean	6.57	1.42	6.27	2.22	5.99	6.03	6.09

Groups I and VII P = 0.014, II and VII P = ∞, IV and VII P = ∞, III and VII, V and VII, VI and VII P not significant.

EXPERIMENTAL RESULTS

Observations on the growth of explants during the time intervals mentioned revealed the following consistent features: signs of growth appeared more rapidly (on the 2nd-4th day) in the case of tumor and normal tissue explants cultured in media with normal, antisera and with sera with a low titer of antitumor (1:20-1:40) and antispleen (1:40) antibodies; when Ehrlich's adenocarcinoma and white mouse spleen tissue were cultured in nutrient media with a high content of antitumor and antispleen antibodies the sign of growth appeared later (4th-6th-8th day).

Since the numerical material collected is vast, only the mean data for each series of experiments, obtained on examination of the preparations on the 10th day of culturing, are given. The data obtained on the 6th-14th day of tissue growth in vitro did not differ substantially from the data obtained on the 10th day.

Table 1 shows the mean ratios of the area of the growth zone to the area of Ehrlich's adenocarcinoma explants in separate series of experiments determined on the 10th day of culturing the tissue in nutrient media containing normal and immune antitumor, antispleen and antihepatic sera of various titers.

As can be seen from Table 1 the same intensity of growth of adenocarcinoma explants was observed when they were cultured in nutrient media containing normal sera, antihepatic sera of low and high titer and antispleen sera of low antibody titer (1:40).

When tumor tissue was explanted on specific antitumor antiserum of low titer (1:20-1:40) some increase of growth was noted in the cultured tissue as compared with the control. When Ehrlich's adenocarcinoma was cultured in antisera with a high titer of antitumor (1:640-1:800) or antispleen (1:800) antibodies the intensity of growth of the explants was markedly lower than in the case of controls (a 4.2-2.7 fold decline).

Data shown in Table 1 were treated statistically by the Fischer-Student method. It was found that the difference between the action of normal serum (control) and anti-Ehrlich's adenocarcinoma sera (titer 1:20-1:40

TABLE 2

Intensity of Growth of White Mouse Spleen Tissue on the 10th Day of Culturing

No. of series	Total no. of explants	No. of explants in each series	Ratio of area of growth zone and area of spleen explant						
			sera						
			antispleen (white mouse)		anti-Ehrlich's adenocarcinoma		antihepatic (white mouse)		Normal nonimmunized
			1:40	1:800	1:20—1:40	1:640—1:800	1:20—1:40	1:360—1:400	
			groups of experiments						
			I	II	III	IV	V	VI	VII
1	1191	99	9.99	1.1	10.7	2.06	—	—	9.7
2		174	8.05	0.92	8.61	3.18	9.47	8.21	7.38
3		153	10.08	1.38	7.99	2.90	8.75	8.14	10.74
4		142	9.47	0.6	8.85	3.80	9.20	8.07	8.73
5		141	10.56	1.07	10.11	3.36	10.61	11.08	9.05
6		154	9.75	1.2	9.45	3.96	8.99	9.40	10.30
7		173	9.95	1.49	9.97	3.98	8.29	8.58	8.99
8		153	8.67	1.13	7.51	1.38	8.83	10.01	10.56
		Mean	9.56	1.11	9.07	3.37	9.16	9.21	9.43

Groups II and VII P = ∞, Groups IV and VII P = ∞, Groups I and VII, III and VII, V and VII, VI and VII P not significant.

and 1:640-1:800) and antispleen (white mouse) serum (titer 1:800) was undoubtedly statistically significant (P = 0.014 and P = ∞). Data obtained on comparison of the same control with the result of the action of antihepatic sera and antispleen (white mouse) serum (titer 1:40) were shown to be statistically nonsignificant (P > 0.05).

Table 2 presents data obtained in separate series of experiments on culturing white mouse spleen tissue for 10 days on nutrient media containing antispleen, antitumor and antihepatic sera of different titer, and also sera of nonimmune rabbits.

It follows from Table 2 that the rate of growth of spleen tissue (white mouse)in vitro in the presence of antihepatic sera of low and high titers, as well as of antispleen and antitumor sera with a low content of specific antibodies (1:20-1:40) is almost the same as the rate of growth of the same tissue in normal sera, used as controls. The differences in mean coefficients in these groups compared with control is found to be statistically nonsignificant when the material is treated by the Fischer-Student method (P > 0.05). When spleen tissue is cultured on nutrient media containing antispleen antisera with a high antibody titer (1:800) a marked growth-inhibiting effect was observed (8.4-fold lowering of the ratios as compared with the control). Explantation of spleen tissue in the presence of antitumor antibodies with titers of 1:640 and 1:800 also caused a reduction in the rate of growth (2.8-fold). The differences between the data of these groups and the control are statistically significant (P = ∞).

Since in all the series of experiments all the ingredients, with the exception of the sera, in the nutrient media were identical, the results obtained suggest a definite relationship between the titer of specific immune antibodies in the nutrient medium and the rate of growth of explants of both tumor and normal tissues. Thus, sera with a high titer of specific antibodies delay the appearance of the manifestations of growth and lower the intensity of growth in explants of the corresponding tissues. Sera with a low titer of antibodies exert no inhibiting effect on the growth of tissues in vitro. Antitumor sera with a low content of specific antibodies even exerted a growth-stimulating effect on Ehrlich's adenocracinoma under our experimental conditions. It must also be taken into account that when there is similarity of antigen structure of the cultured tumor and normal tissues (e. g. tissue of Ehrlich's adenocarcinoma and white mouse spleen [1, 3]) antisera immune with respect to them and possessing a sufficiently high antibody titer may exert a growth inhibiting cross effect on the tissues being cultured.

SUMMARY

The tissue of subcutaneous form of Ehrlich's adenocarcinoma (explant 1284) and the spleen of a white mouse (explant 1191) were cultured in vitro on nutrient media containing normal or immune antitumor, antisplenic and antihepatic sera of low and high titers. The results of this work demonstrate that sera with high titer of specific antibodies retard the appearance of manifestations of growth and affect its intensity in the explants of corresponding tissues. Low titer sera do not possess these properties. On the contrary, antitumor sera with a low content of specific antibodies have a growth stimulating effect on the tissues of Ehrlich's adenocarcinoma.

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THE RELATIONSHIP BETWEEN THE TITER OF TUMOR ANTIBODIES AND THE CHARACTER OF THE ANTIGENIC STIMULUS

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The production of immune sera with a relatively high titer of antibodies against tumors is of great scientific and practical importance. Several investigations carried out in this direction demonstrate that the production of immune antibodies depends, on the one hand, on the species and individual characteristics of the experimental animals undergoing immunization [1, 2, 3], and on the other hand, on the character of the antigenic stimulus itself. For instance it was established by the investigations of Horn [4] that sera with a high antibody titer are obtained by immunization of rabbits with whole ascitic cells from a carcinomatous tumor, but not by saline extracts of the separate fractions of these same cells. In this connection it was shown that a serum of the first type had a comparatively high titer (1280) in the agglutination reaction, whereas sera of the second type, produced by immunization of rabbits with saline extracts of the nucleoprotein fraction of ascitic cells, were in general inactive in this reaction. It is also well known that hemolytic sera, used in the complement fixation reaction, are obtained with a high antibody titer during immunization of rabbits with whole sheep's red cells, but not with a saline extract of these cells.

TABLE 1

Scheme of Immunization of Rabbits with Saline Extracts of Subcutaneous Forms of Tumors and Whole Ascitic Cells from the Same Tumors

Form of antigen of tumor tissue	Injections				
	1st	2nd	3-rd	4-th	5-th
	volume of antigen injected (in ml)				
Saline extract	1.0	1.0	1.5	1.5	2.0
Whole ascitic cells	1.0	1.0	1.5	1.5	2.0

In view of these findings we decided to study the relationship between the titer of antibodies against tumors and the character of the antigenic stimulus of the same origin.

EXPERIMENTAL METHOD

In the immunization experiments 20 sexually mature male rabbits, aged from 12 to 18 months, were used. The material used for immunization was two transplanted malignant tumors: an Ehrlich's mouse adenocarcinoma and a Iosida's rat sarcoma. In the experiments one group of rabbits was immunized with saline extracts of subcutaneous forms of the tumors and another group with whole ascitic cells from the same tumors.

TABLE 2

Complement Fixation Reaction of Sera Against Ehrlich's Mouse Adenocarcinoma with Homologous Antigen

Dilution of sera	Sera obtained by immunization of rabbits with saline extracts of a subcutaneous form of Ehrlich's mouse adenocarcinoma									Sera obtained by immunization of rabbits with whole ascitic cells of Ehrlich's mouse adenocarcinoma								
	no. of sera									no. of sera								
	5	870	881	971	102	154	148	4510	1479	3135	1015	1516	1518	309	1473	1004		
1:20	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++		
1:40	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++		
1:80	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++		
1:160	++++	++	+	++	+	+	+	+	+	+	+	+	+	+	+	+		
1:320	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
1:640	+	-	-	+(+)	-	+(+)	+	-	+	+	+	+	+	-	-	-		
1:1280	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-		
1:2560	-	-	-	-	-	-	-	-	-	+(+)	+	+	+(+)	+	-	-		
1:5120	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		

The saline extracts of the subcutaneous forms of the tumors were prepared as follows. A subcutaneous tumor transplantate 7-10 days old was extracted from the experimental animals and ground in a mortar in sterile conditions for 10 minutes.

The ground tumor tissue was suspended in physiological saline in a proportion of 1:20, i. e. 0.5 g of tumor tissue was suspended in 10 ml of physiological saline. This suspension of tumor tissue was allowed to stand in the incubator for one hour at a temperature of 37°C, after which it was kept in the refrigerator at 4°C for 24 hours.

Before immunization the suspension of tumor tissue was centrifuged for 15 minutes at 2500 rpm.

The whole ascitic carcinoma cells were taken from the experimental animals on the 6th-8th day after inoculation, and separated from ascitic fluid by weak centrifugation for 5 minutes. Next they were washed twice with physiological saline and suspended in this solution. Before immunization the number of ascitic cells in 1 ml of the cell suspension in physiological saline was measured in a Goriaev counting chamber. In the experiments ascitic fluid which was free from blood was used, containing only ascitic carcinoma cells as its basic component.

The rabbits were immunized by five intravenous injections according to the following scheme (Table 1).

The number of ascitic cells in 1 ml of physiological saline used for immunization varied in the case of Ehrlich's mouse adenocarcinoma from 100 to 200 million, and in the case of Iosida's rat sarcoma, from 50 to 100 million. Injection of tumor antigens into the rabbits was carried out every 2 days for 3 injections, and an interval of 7 days was left between the 3rd and 4th injections. Serum was taken from the rabbits on 10th day after the last injection, and it was taken from the heart. Before the experiment was set up, the sera for testing were heated to a temperature of 56°C for 30 minutes. Next these sera were tested in the complement fixation reaction in order to determine the presence of antitumor antibodies in them, and their specificity. The antigens used in the serological reactions were saline extracts of subcutaneous forms of tumors and of tissues from the liver and spleen of the corresponding species of animal. The antigens

TABLE 3

Complement Fixation Reaction of Sera Against Iosida's Rat Sarcoma with Homologous Antigen

Dilution of sera	Sera obtained by immunization of rabbits			
	with a saline extract of a subcutaneous form		with whole ascitic cells	
	of Iosida's rat sarcoma			
	no. of sera			
	3643	3095	2906	3730
1 : 20	++++	++++	++++	++++
1 : 40	++++	++++	++++	++++
1 : 80	+++	++++	++++	++++
1 : 160	++	+++	++++	++++
1 : 320	+(+)	++	++++	++++
1 : 640	+	+(+)	++++	++++
1 : 1280	Г	Г	+++	+++
1 : 2560	Г	Г	+(+)	+

were prepared in proportions of 1:10. Before the main experiment was set up all these antigens were titrated and a working dose was estimated for them. The complement fixation reactions were performed in the usual way at a temperature of 37°C, with provision of suitable controls. In the experiment the only reactions to be considered were those in which the same results were obtained on two occasions.

EXPERIMENTAL RESULTS

In the immunization experiments on rabbits with antigens from tissue of an Ehrlich mouse adenocarcinoma 16 experimental animals were used, of which 9 were immunized with a saline extract of a subcutaneous form of the mouse tumor and 7 with whole ascitic cells of the same tumor. The results of these experiments are shown in Table 2.

Of the 9 rabbits immunized with a saline extract of tissue of a subcutaneous form of Ehrlich's mouse adenocarcinoma, in 4 the sera were found to have a low titer of anticarcinoma antibodies. The sera of rabbits Nos. 870 and 881 reacted with antigen from the tumor tissue in a dilution of 1:160 to + +, but sera of rabbits Nos. 102 and 4510 reacted with the same antigen in a dilution of 1:80 to + +. The serum of only one rabbit (No. 154) reacted with tumor antigen in a comparatively high titer: in a dilution of 1:1280 to + (+).

A somewhat different picture was obtained during immunization of rabbits with whole ascitic cells of Ehrlich's mouse adenocarcinoma. The sera of these rabbits reacted with tumor antigen in a comparatively high titer. The sera of rabbits Nos. 1015, 1516 and 309 reacted in dilution of 1:2560 with antigen from the tumor tissue to + + and + + +, but sera of rabbits Nos. 3135, 1518, 1473 and 1004 showed a titer of tumor antibodies corresponding to a dilution of 1:1280.

Thus sera against Ehrlich's mouse adenocarcinoma had a higher titer of tumor antibodies after immunization of rabbits with whole ascitic carcinoma cells than with saline extracts of a subcutaneous form of the tumor.

A similar picture was likewise observed after immunization of rabbits with antigens from tissue of Iosida's rat sarcoma. As may be seen from the figures given in Table 3, the sera of rabbits Nos. 3643 and 3095, obtained by immunization of experimental animals with a saline extract of tissue of a subcutaneous form of Iosida's rat sarcoma, had a comparatively low titer of tumor antibodies. These sera reacted with antigens from tumor tissue in a dilution of 1:320 to + + and + (+), whereas the sera obtained by immunization of rabbits with whole ascitic cells of the same tumor reacted with tumor antigens in higher dilutions (1:280 to + + + and + + + +).

By the example of the two inoculated malignant tumors it is clear that during immunization of rabbits the strongest antigenic stimuli are not saline extracts of the tissues of subcutaneous forms of tumors, but whole ascitic cells.

TABLE 4

Complement Fixation Reaction of Sera Against Tumor Tissues with Homologous Antigens and with Antigens from Tissues of Normal Organs of the Corresponding Species of Animal

Dilution of sera	Sera obtained by immunization of rabbits											
	with a saline extract from Ehrlich's mouse adenocarcinoma			with whole ascitic cells from Ehrlich's mouse adenocarcinoma			with a saline extract from Iosida's rat sarcoma			with whole ascitic cells from Iosida's rat sarcoma		
	antigens from tissue of the											
	tumor	liver	spleen	tumor	liver	spleen	tumor	liver	spleen	tumor	liver	spleen
1:20	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
1:40	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
1:80	++++	-+++	++++	++++	+++-	++++	++++	++++	++++	++++	++++	++++
1:160	++++	+++	++++	++++	++++	+++-	+++	+++	++++	++++	++++	++++
1:320	++++	++	+++	++++	++++	++++	++	+(+)	++	++++	++++	++++
1:640	+++	r	++	++++	+++-	+++-	+(+)	r	+	++++	+++	+-++
1:1280	+	r	r	++++	r	+++	r	r	r	++++	+(+)	+++
1:2560	r	r	r	2+	r	+	r	r	r	+(+)	r	+(+)

In the next experiment we tested the specificity of the antitumor sera. For this purpose we performed the complement fixation reaction simultaneously with antigens from tumor and normal tissues (liver and spleen). In Table 4 is shown a selection of results of certain of these reactions.

The experiment showed that the test sera possess a low degree of specificity since they reacted at a comparatively high titer with antigens from both tumor and normal tissues of the experimental animals. This is evidently explained by the fact that whole ascitic malignant cells include in their composition a large number of antigens common to the species, which are present in the normal organs and tissues of the particular species of animal.

SUMMARY

Serums with a comparatively high titer of immune antibodies were obtained in immunization of rabbits by ascitic cells of malignant tumors (Ehrlich's adenocarcinoma of mice and Iosida's sarcoma of rats). Serums with lower titers of immune antibodies were recovered in immunization of rabbits by the water-salt extracts of the tissue of subcutaneous forms of tumors. Serums of the first, as well as of the second type are not very specific: they react in comparatively high titers with the antigens from the tumor tissues and with those from the tissues of normal organs of the laboratory animals.

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THE SIMPLIFICATION OF THE ANTIGENIC STRUCTURE OF LIVER PROTEINS DURING EXPERIMENTAL CARCINOGENESIS

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During the last decade Zil'ber et al. [2, 14] demonstrated the presence in tumors of specific antigens, not found in normal tissues. The most important technic in the domonstration of these tumor antigens was the application of the anaphylactic reaction with desensitization in guinea pigs.

The application of this technic to the study of the antigenic composition of livers during carcinogenesis brought to light a number of new facts. Thus, it was possible to demonstrate the presence of an antigen with the specificity of orthoaminoazotoluene (OAAT) in the early stages of action of this compound [3]; a specific antigen in induced and transplanted hepatoma was found [4, 5, 1]; an antigen similar to that of hepatoma was also identified in the precancer stage [1]. At the same time there appeared reports dealing with the simplification of tissue antigen structure in the process of carcinogenesis. Such results were claimed by Weiler [11-13], who employed complement fixation, for liver tumors in rats and kidney tumors in hamsters. Seligmann, Grabar and Bernard [10] employed precipitation in gels to demonstrate the simplification of antigenic structure in leukemia cells compared to normal leucocytes. Using the same technic, Parnes and Abelev [7] found simplified antigenic structure in tissues affected by leucosis.

The present work deals with the demonstration of simplified antigenic structure in the liver during experimental carcinogenesis, using the anaphylaxis and desensitization technic.

EXPERIMENTAL METHODS

Guinea pigs were used throughout. The antigens used were protein fractions, obtained from the tissues investigated by extracting in slightly alkaline (ca. pH 8) water, rejecting the fraction which precipitated on subsequent acidification to pH 6, and collecting the fraction which precipitated when pH was further lowered to 4.5. A batch of 4-5 mice was used in each antigen preparation. Guinea pigs were sensitized by a subcutaneous injection of from 3.5 to 10 mg of antigen protein from livers of normal C_3HA strain mice. The protein content was determined by the Kjeldahl method, in the analytical laboratory of this Institute (head of laboratory: A. D. Chinaeva). In order to eliminate possible differences in experimental results due to the different blood content of hepatomas and normal livers, some of the organs were perfused with physiological saline prior to excision. Desensitizing agents were introduced intravenously 1.5-2 months after sensitization. The antigens used in desensitization were prepared by the method described above, from livers of mice which were receiving periodical paintings with a 1% solution of OAAT in benzene over a period of 2-2.5 months (pre-cancer stage); livers from animals bearing OAAT-induced hepatoma, or a transplanted hepatoma, were also used. Protein from livers of normal C_3HA mice was introduced as a resolving antigen.

All antigens were checked prior to experiment for possible toxicity, by intravenous injection into normal

TABLE

A Comparison of Normal Liver Antigens With Those of Precancerous Liver and Hepatoma

	Sensitization			Desensitization (check for completeness)				Resolving introduction						
	normal liver antigen	number of guinea pigs	dose, mg protein	Antigen		dose, mg protein	reaction	normal liver antigen	dose, mg protein	no. of guinea pigs which gave reaction				
										—	+	++	+++	++++
1	№ 97	4	6.77	№ 94	Mouse liver in the precancer stage (after 3 mos of painting with OAAT)	7.4—9.2	—	№ 976	7—9	—	2	2	—	—
2	№ 113	5	3.5—6.3	117		7.2—8.5	—	№ 124	7—8.5	1	3	1	—	—
3	№ 159 after perfusion	5	7.7—10.2	176		7.6—10.0	—	№ 159 after perfusion	7.6—9.4	—	1	4	—	—
4	№ 97	4	6.77	№ 95	Induced malignant hepatoma	8.8	—	№ 976	9.1	—	4	—	—	—
5	№ 167 after perfusion	5	7.57	161		7.53	—	№ 159 after perfusion	7.5	—	1	4	—	—
6	№ 124	6	3.4—4.2	123в	Induced non-malignant hepatoma	4.3	—	№ 127	4.3	1	3	2	—	—
7	№ 144	5	6,2	154		6.5	—	№ 144	6.5	—	4	1	—	—
8	№ 136 bearing transplanted hepatoma	6	5.5	135	Transplanted hepatoma	5.5	—	№ 136 bearing transplanted hepatoma	5.5	—	2	4	—	—
9	№ 144	5	6.2	152	as above	3.6—6.2	—	№ 144	3.7—6.2	—	—	4	1	—
10	№ 148	5	8.3	156	» »	8.8	—	№ 153	8.9	—	1	2	2	—
11	№ 157 after perfusion	5	7.5	160	» »	7.5	—	№ 157 and 159 after perfusion	7.5	1	2	2	—	—
12	№ 159 after perfusion	3	6.8—8.3	167	» »	6.8—8.5	—	№ 159 after perfusion	6.8—8.5	—	—	1	2	—
13	№ 173 after perfusion	2	5.1	189	» »	9.5	—	№ 190 after perfusion	2.5	—	2	—	—	—
		3				9.5		№ 190	4.9	—	—	2	—	1
	Total number of guinea pigs used	63	—	—	—	—	—	—	—	3	25	29	5	1

guinea pigs. No reaction to the antigens was observed in any case with these animals. A total number of 13 experiments were carried out with 63 guinea pigs, 12 various normal antigens being tested.

EXPERIMENTAL RESULTS

The results have been presented in the Table. It will be seen that in 3 experiments (14 guinea pigs) the antigens used were obtained from livers at the pre-cancer stage, in 4 other experiments (20 guinea pigs) they were obtained from induced hepatoma, and in 6 others (29 guinea pigs) – from transplanted hepatoma. As a rule, the guinea pigs reacted to the resolving introduction of normal liver proteins with an anaphylactic reaction of a strength + or ++. When compared with transplanted hepatoma, guinea pigs responded, in some cases, with a reaction of strength +++, or even ++++ (death). The results were not affected by the source of the antigen, and both the sensitizing and resolving antigens could be derived from different groups of mice. Thus, no difference could be detected between the results of experiments 2, 5, 6, 10 and 13, where antigens from a variety of preparations were used, and those of experiments 1, 3, 4, 7, 8, 9 and 12, where the same antigens were used throughout. The blood content of the various preparations was also without any apparent effect on the results. In experiment 10, during desensitization, and prior to the introduction of the antigen from transplanted hepatoma, the guinea pigs were given two lots of 6 mg each of serum proteins from hepatoma-bearing mice. In experiments 3, 5, 11, 12 and 13 the livers of the normal mice were perfused with physiological saline prior to preparation of the antigens; nevertheless, the results obtained in this experimental series did not differ from those in other corresponding groups.

It is noteworthy that antigenic differences between normal liver and transplantable hepatoma were observed only in cases when the material was obtained from the same animals (Experiment 8). Positive results were obtained in experiment 13, where the resolving antigen was added in doses 2 and 4 times lower than those employed in the test of completeness of desensitization.

This seems to indicate that the situation did not simply involve quantitative differences in the content of normal components in hepatoma and normal livers.

The results thus show that the normal mouse liver contains antigens (or antigen) which cannot be demonstrated in livers of mice bearing hepatoma, whether induced or transplanted, or in mouse livers in the pre-cancer stage.

The antigenic simplification of tumor tissue may be contemplated as the immunological demonstration of its well known biological and morphological de-differentiation; it may well prove to be an important link in the pathogenesis of a malignant growth. The importance of this phenomenon may be better realized in the light of the recently advanced "exclusion theory" of Miller and Miller [6], which has been supported by other authors [8, 9]. On the basis of the observed presence in the precancer liver of a protein-bound dye, and the absence of bound dye in hepatoma, Miller and Miller advanced a working theory, consisting of the following:

In the process of carcinogenesis the azo dye – paradimethylaminoazobenzene – is bound by proteins which play an important role in "the reaction of the cell to the action of growth-regulating intracellular factors". Successive cell generations contain decreasingly smaller amounts of this protein until, finally, cells are formed which are completely devoid of growth-regulating proteins. These cells respond with uninterrupted division to the continuous flow of nutritive substances, and finally become malignant.

Considering the fact that the tumors are devoid of certain antigens which are present in normal tissues, and that they contain specific tumor antigens which normal tissues do not contain, it is feasible that in the process of carcinogenesis there takes place not merely a loss of normal proteins, but a complex transformation of the antigenic structure of the tissue.

Some antigens (and consequently, proteins) disappear while others appear. It is difficult to state, on present evidence, whether the process involves a transformation of one protein, or a complete substitution of an old protein by the new one. It is even difficult to determine which antigen is expended in the simplification of the tissue structure of the tumor. For instance, Weiler [11], on the basis of his experimental evidence, concluded that both hepatoma and kidney tumor lost their tissue-specific antigen. It is the present author's opinion that the solution of this problem requires further investigation with the application of various immunological methods.

SUMMARY

. The simplification of the antigenic structure has been revealed during the experimental carcinogenesis in mouse liver at the precarcinogenic stage, induced with orthoaminoazotoluene in transplanted and induced hepatoma. These experiments were performed with the aid of the anaphylactic reaction following desensitization.

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ONCOLOGY

THE ANTITUMOR ACTIVITY OF THE OPTIC ISOMERS OF SARCOLYSIN

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As has already been reported, the drug sarcolysin, which is DL-n-di-(2-chloroethyl) aminophenylalanine, was created according to the principle of attachment of active chemical groups to a metabolite which plays a leading part in the metabolism of tumor tissue [1]. In this particular case the chloroethylamine group was

combined with the unsubstituted amino acid DL-phenylalanine. Experimental research carried out on the compound thus obtained demonstrated its high antitumor acticity, which is more powerful than any of the other compounds of the chloroethylamines [3]. The presence of a natural component in the sarcolysin molecule called for investigation to ascertain the importance of its natural portion in the mechanism of the antitumor action of the compound.

TABLE 1

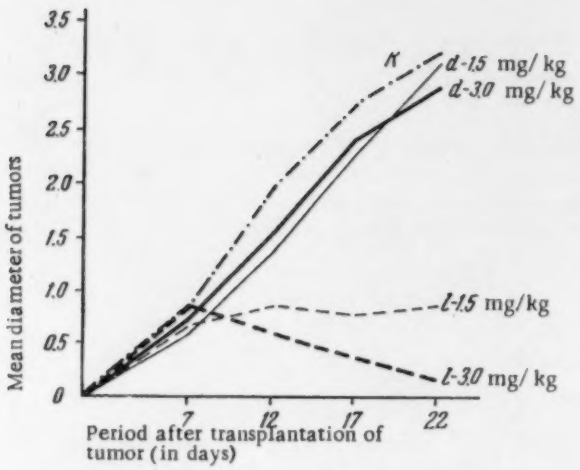
The Toxicity of Optical Isomers of Sarcolysin

Preparation	Dose, in mg/kg	No. of animals	No. of animals dying	% dying	Time of death in days
L-sarcolysin	25	10	9	90	4—6
	20	10	6	60	4—6
	15	10	3	30	4—6
	10	10	0	0	—
DL-sarcolysin	30	10	10	100	
	25	10	6	60	4—7
	20	10	4	40	4—7
	15	10	0	0	—
D-sarcolysin	35	10	10	100	3
	30	10	9	90	3
	25	10	6	60	3

TABLE 2

The Action of Optical Isomers of Sarcolysin on the Growth of Sarcoma 45

Experiment no.	Preparation	group	No. of animals	Dose in mg/kg (numerator and intervals between injections in hr. (denominator)	Mean wt. of tumor at the end of treatment (in g)	% retardation	% resolution
48	DL-sarcolysin	1	10	3/72	0.25	99.5	70
	as above	2	10	1.5/72	11.1	78.9	0
		3	10	0.75/72	24.7	53	0
	L-sarcolysin	4	10	3/72	0.09	99.8	70
	as above	5	10	1.5/72	1.95	96.2	30
		6	10	0.75/72	13.5	75	0
	Control	7	10	—	52.6	—	0
50	DL-sarcolysin	1	10	3/72	0.45	98.7	40
	as above	2	9	1.5/72	8.8	74.3	0
	L-sarcolysin	3	9	3/72	0.28	99.2	44
	as above	4	10	1.5/72	1.04	96.9	20
	D-sarcolysin	5	10	3/72	22.8	33.5	0
	as above	6	10	1.5/72	28.4	14.3	0
	Control	7	10	—	34.4	0	10



Growth of the tumors under the influence of optical isomers of sarcolysin. Treatment with D-sarcolysin (═══), treatment with L-sarcolysin (- - -), K — control.

The growth of tumors is known to be connected with intensive syntheses of protein, in the process of which amino acids are used up. It might therefore be suggested that sarcolysin, being a derivative of an unsubstituted amino acid, might enter into competetive relations with the natural unsubstituted amino acids and interfere with the syntheses of proteins, i. e. act according to the principle of an antimetabolite, especially as there are indications in the literature of the greater or lesser expression of antitumor action of the amino acid antagonists [2, 6, 9].

Another possible explanation was that the phenylalanine in the sarcolysin molecule may act as a "conductor" of the chemically active chloroethylamine group in the tumor tissue.

Sarcolysin, having one asymmetrical carbon atom in the alanine part of the molecule just like phenylalanine, may exist in the form of 2 optical isomers: the L-form of phenylalanine is natural, the D-form does not occur in nature.

If the hypotheses on which the creation of sarcolysin is based is correct, it would be expected that the laevo, natural isomer would possess the more pronounced biological activity.

In the papers by the British authors Bergel and Stock [4, 5] reference is made to Haddow's findings that the laevo isomer possesses greater antitumor activity. The different antitumor activity of the optic isomers is also mentioned in the paper by Koller and Veronesi [7].

In the laboratory of chemical syntheses of the Institute of Experimental Pathology and Therapy of Cancer of the AMN SSSR optical isomers of sarcolysin* have also been prepared, which enabled us to proceed to evalu-

* E. N. Shkodinskaia and O. S. Vasina (L-form) and I. S. Levi (D-form).

ate the role of the natural portion of the molecule of sarcolysin.

For this purpose in the present investigation a comparative study was undertaken of the toxicity and the antitumor activity of the D- and L-forms of sarcolysin.

EXPERIMENTAL METHOD

The investigation of the toxicity of the optical isomers of sarcolysin was carried out on 110 white rats weighing 100-120 g, and the investigation of the antitumor activity on 128 rats with a transplanted sarcoma 45.

The preparations were dissolved in physiological saline, heated preliminarily to 50-60° and injected intraperitoneally into the experimental animals. In the experiments to determine the toxity the compounds were injected once, but in the experimental treatment of sarcoma 45 they were injected repeatedly at intervals of 72 hours. Treatment continued for 21 days. Treatment began on the 7th-8th day after transplantation, i. e. while the tumors were measurable and weighed on the average of 0.7-0.8 g. The dosage of the compounds was calculated in milligrams of the dry compound per kg body weight of the animal.

In estimating the toxicity of the preparations we took into consideration the number of animals dying and the time of death. In estimating the antitumor activity attention was directed firstly, to the difference between the mean weight of the tumors in animals treated with the preparations and in the control animals, i. e. untreated (calculated as percentage retardation*), and secondly, to the changes in the growth of the tumors in animals of the same groups as determined by measurement of the tumors every fifth day (length, width, height), from which the mean diameter of the tumors was calculated.

The investigation of the toxicity and antitumor activity of the optical isomers of sarcolysin was carried out in comparison with the racemate.

EXPERIMENTAL RESULTS

In Table 1 are given the results of the experiments to determine the toxicity.

It can be seen from Table 1 that there is no sharp difference between the toxicity of L-sarcolysin and that of D-sarcolysin. The toxicity of the D-form is practically equal to that of the racemate L-form, being slightly greater than that of the latter. Thus the dose of DL-sarcolysin and D-sarcolysin which causes death of 60% of the animals is 25 mg/kg, whereas the corresponding dose of L-sarcolysin is 20 mg/kg.

The D- and L-forms are sharply distinguished from each other in their antitumor activity (Table 2): the activity of the L-sarcolysin in a dose of 3 mg/kg** is equal to the activity of the racemate and in smaller doses actually exceeds it; the D-form possesses only weak antitumor activity.

The difference in the antitumor activity of D- and L-sarcolysin is illustrated by the diagram in which are shown graphically the growth of the tumors and its changes under the influence of the optical isomers of sarcolysin in doses of 3 and 1.5 mg/kg, injected at intervals of 72 hours.

As can be seen from the Figure, the growth of the tumors during the action of D-sarcolysin on them proceeds almost parallel to the growth of the tumors in the control group of animals. Growth of the tumors under the action of L-sarcolysin is considerably retarded to an extent which depends directly on the dose.

The investigations carried out showed that the optical isomers of sarcolysin, which are quite similar in their chemical properties, differ in their biological activity.

The hypotheses put forward by Haddow, that the dextra isomer is more rapidly destroyed in the body as a result of the greater activity of D-amino acid oxidase, was not confirmed by experimental trial. Thus at the present time the most likely explanation of the difference in the antitumor activity of the optical isomers is their stereochemical structure.

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* The percentage retardation was calculated from the formula:

$$\frac{\text{Mean weight of tumors in control animals} - \text{Mean weight of tumors in experimental animals} \cdot 100}{\text{Mean weight of tumors in control animals}}$$

** This dose is slightly below the optimal dose for sarcolysin; the latter is 5 mg/kg when injected at intervals of 72 hours.

G. L. Zhdanov showed in our laboratory that the isomers of sarcolysin differ only slightly from each other in their action on the blood. They cause roughly the same degree of suppression of hemopoiesis, although the depression due to injection of the L-form is more lasting than that due to the D-form of sarcolysin.

On the basis of all the data it can be suggested that the slight differences in the toxicity and the action on the blood, in contrast to the great difference in antitumor activity, are explained by differences in the mechanism of action of sarcolysin on tumor and normal tissues. However this question demands further study. The fact that L-sarcolysin, possessing the natural configuration of the molecule, was found to be more active than the D-form of sarcolysin makes the hypotheses on which is based the creation of the antitumor preparation sarcolysin a probable one, and strengthens us in the opinion that the natural portion of the sarcolysin molecule plays a definite role in the mechanism of its antitumor action.

Further evidence in favor of this view is provided by the experiments which we performed to ascertain the influence of the structural analogues of sarcolysin on its antitumor activity.

Thus besides the racemate, L-sarcolysin can be recommended for clinical trial as an active antitumor preparation.

SUMMARY

Investigations were conducted on rats with transplantation of sarcoma 45. These experiments in coordination with literature data, demonstrated that optical isomers of the antitumor preparation sarcolysin, i. e. DL-p-di-(2-chloroethyl) aminophenylalanine, although being similar by their toxic properties, were different in their biological activity. Considerable antitumor activity of L-form of sarcolysin, possessing a natural configuration of the molecule, in difference from an unnatural L-form confirms the belief that metabolite in the sarcolysin molecule plays a definite role in the mechanism of the antitumor effect of this preparation.

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THE DEVELOPMENT OF TUMORS OF THE MAMMARY GLANDS IN RATS

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There are indications in the literature of the frequent (up to 36%) appearance of fibroadenomas of the mammary glands in old virgin rats [1].

While studying destructive changes in the liver produced in rats by chronic poisoning with carbon tetrachloride, we observed in many experimental animals the development of tumors of the mammary gland. These tumors were subjected to histological examination. The results of this investigation form the subject of the present communication.

EXPERIMENTAL METHOD

The experiments were carried out on virgin female white rats, one year old, with an initial body weight of 230-270 g. Sixty-five animals were used in the experiment, of which 40 were experimental and 25 control. The experimental rats were injected subcutaneously twice a week with 0.2 ml of CCl_4 for a period of $9\frac{1}{2}$ months.

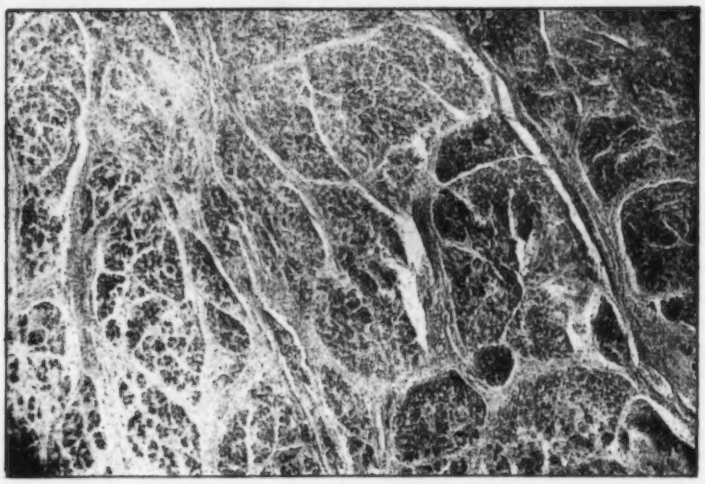

Fig. 1. A young tumor developing in a rat after 5 months 18 days of injections of CCl_4. Stained with hematoxylin-eosin. Magnification: ocular 6 ×, objective 8.

Fig. 2. Fibroadenoma. Proliferation of connective tissue. Presence of alkaline phosphatase (Gomori's method). Magnification: ocular 8 ×, objective 20.

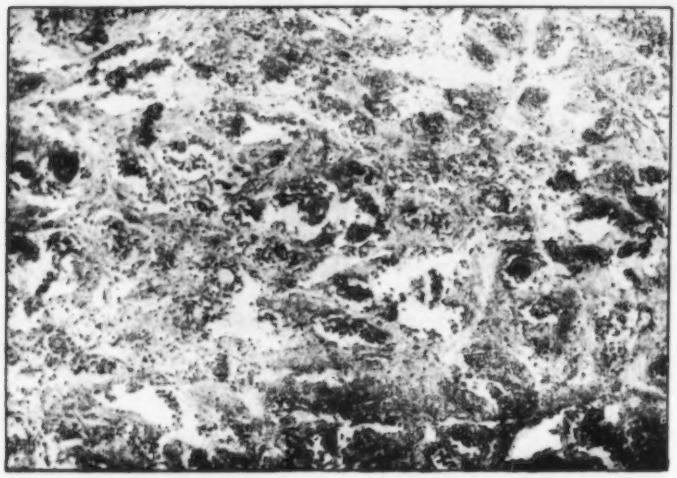

Fig. 3. Fibroadenoma. Presence of alkaline phosphatase (by Gomori's method). Magnification: ocular 8 ×, objective 12.

After this period of time had elapsed the injections of CCl_4 were discontinued and the rats were kept in their cages on an ordinary balanced diet. Throughout the whole period of the experiment the control rats received no injections. The experiment lasted 375 days. Experimental rats were killed at different times which were determined by the development of the tumor. The tumor was freed from surrounding tissues and weighed. Pieces of tumor were fixed in 80° alcohol and in Carnoy's fluid. The material was embedded in paraffin wax by the usual method. Sections, 8-10 μ in thickness, were stained with hematoxylin-eosin and by Mallory's method. The alkaline phosphatase was estimated by the Gomori (1939) method; ribonucleic acid (RNA) was detected by staining the sections with methyl green-pyronin by Unna's method.

EXPERIMENTAL RESULTS

During our observations on the experimental animals we observed the development of a tumor of the mammary gland in 24 of the rats (60%) which were given injections of CCl_4. The tumor was usually localized to the region of the superior or posterior limbs (2nd or 5th pairs of nipples).

The first tumor appeared in rat No. 15 after 5 months 18 days of injections of CCl_4. The animal was killed; it weighed 230 g. The tumor, developing in the region of the right forelimb (2nd pair of nipples), was the size of a large walnut; it was easily separated from the surrounding tissues, and weighed 23 g. The tumor was firm, pinkish white in color, and cut with difficulty with scissors. On section white nodules could be seen, of different sizes and resembling cartilage.

Histological study of sections of the "young" tumor showed that the glandular tissue was preserved to a considerable extent, but many lobules had undergone degenerative changes. The lobules were connected by broad bands of coarse, fibrous connective tissue (Fig. 1). In isolated areas of the gland there appeared a large amount of coarse, hyalinized connective tissue, among which was preserved a very insignificant amount of glandular epithelium.

In the remaining 23 animals tumors developed in the 10th-12th month after the beginning of the experiment. The tumors grew slowly and were readily separated from surrounding tissues. The rats were killed at different periods of growth of the tumor. The weight of the tumors varied from 21 to 134 g. The "young" tumors were the size of pigeons' eggs and consisted of dense, white tissue, which cut with difficulty with the scissors; foci of necrosis were not present in these tumors. The "old" tumors reached the size of an apple, contained areas of hemorrhage, were soft to the touch, and on section they yielded milk, mixed with blood and pus. The lactiferous ducts were dilated (Fig. 2). Foci of necrosis in the tumor were mingled with areas of dense connective tissue. The blood vessels were markedly hyperemic. Inflammatory foci were encountered; in these cases the appearance of a large number of lipoid cells was observed.

Determination of the content of alkaline phosphatase and RNA showed that the nuclei of the epithelial cells of the alveoli were characterized by considerable enzymic activity; the nucleoli were rich in RNA, and the cytoplasm contained a small quantity of enzyme and RNA. The cytoplasm of some epithelial cells of the alveoli were intensely vacuolized; no enzyme nor RNA was observed in the vacuoles. The secretion in the lumen of the terminal sections was rich in alkaline phosphatase and RNA. In the connective tissue only the nuclei gave a positive reaction for alkaline phosphatase (Fig. 3).

In the control rats the development of a tumor of the mammary gland was observed in only one case (4%).

Macro- and microscopic examination showed that the tumor developing in the mammary gland of the experimental and control rats is a fibroadenoma. We believe that the prolonged action of injections of CCl_4 creates favorable conditions for the formation of a fibroadenoma of the mammary gland in rats.

SUMMARY

Fibroadenoma of the mammary glands appeared in 24 of the 60 rats which received subcutaneous injections of carbon tetrachloride for $9\frac{1}{2}$ months. It is assumed that prolonged administration of this chemical creates favorable conditions for development of tumors of the mammary gland.

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EXPERIMENTAL BIOLOGY

PROLIFERATION OF THE EPITHELIUM OF THE MAMMARY GLANDS IN VARIOUS STAGES OF THE SEXUAL CYCLE

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The study of the functional morphology of the mammary glands of laboratory animals, especially mice, is one of the current tasks of experimental oncology.

The mammary gland is an organ which, in female animals, is exposed to continual changes on the attainment of sexual maturity, with the interchanging phases of the sexual cycle, the occurrence of pregnancy and the period of lactation and, finally, on the cessation of the reproductive functions in old age.

The revelation of the laws governing the physiological proliferation of the epithelium of the mammary glands must help in the discovery of the causes of pathological proliferation, leading to the appearance of benign and malignant tumors of these organs.

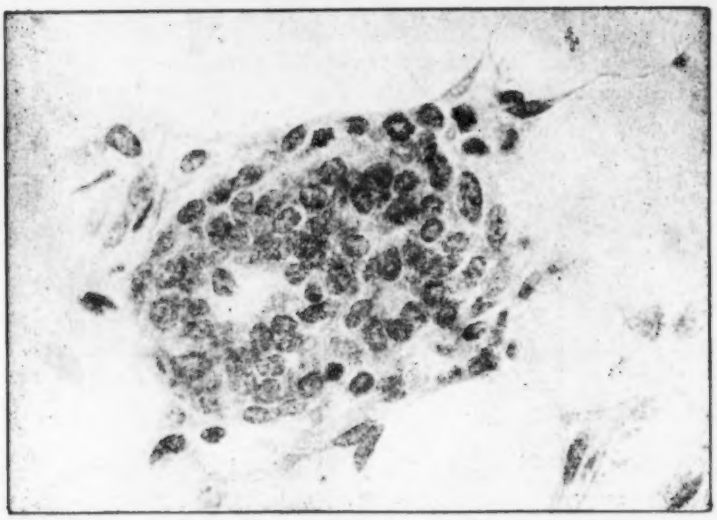

Fig. 1. A proliferative epithelial nodule in the mammary gland of a white mouse. Proestrus. Magnification 500 times.

Fig. 2. Death of surplus epithelial cells in the mammary gland of the white mouse. Diestrus. Desquamation of epithelial cells into the lumen of an efferent duct. Magnification 500 times.

Fig. 3. Infiltration of an epithelial nodule with leucocytes. Metestrus. Magnification 500 times.

One of the most objective methods of study of the proliferation of cells is the method of determination of mitotic activity. However, despite the large number of investigations of the morphology of the mammary glands [2, 5, 6, 7] we were able to find only one work devoted to the study of the mitotic activity of the epithelium of the mammary glands of the mouse [3]. Bullough, the author of this paper, investigated the changes in the mitotic activity of the epithelium of the small ducts of the mammary glands of mice in the various phases of the sexual cycle. He found that in the course of the sexual cycle there are two periods of increase in the number of cell divisions: the first – at the end of diestrus, and the second – at the end of "full" estrus with a maximum after ovulation. The mitotic activity of the epithelium of the mammary glands during pregnancy and lactation has been studied in cows and rats by A. D. Al'tmas [1]. He came to the conclusion that mitotic activity is high only during pregnancy, whereas during lactation, when the number of mitoses is insignificant, proliferation of the cells is brought about by amitosis.

In the present investigation we set out to study the changes in mitotic activity of the epithelium of the terminal portions of the mammary glands in mice during the different stages of the sexual cycle, and also to trace the morphological changes in the glands which correspond to these stages.

EXPERIMENTAL METHOD

Experiments were carried out on female white mice, of mixed strains, aged roughly 4-5 months. In order to determine the stage of the sexual cycle the method of vaginal smears was used. At each stage of the cycle 10 animals were killed (by decapitation). The animals were killed at 12 noon. The second right thoracic mammary gland was fixed in Zenker's solution, and embedded in paraffin wax. Sections were cut to a thickness of 7 μ and stained with hematoxylin by Caracci's method. Every other section was examined under the oil-immersion system of the microscope, and 6000 cells of the terminal portions were counted, records being made of all mitoses, beginning with early prophase and ending with late telophase. The mitotic activity was expressed as the number of mitoses per thousand epithelial cells. The statistical significance of the differences in mitotic activity at the various phases was determined by the Fisher-Student method.

EXPERIMENTAL RESULTS

The mammary gland of sexually mature virgin female mice consists of a common outlet duct of the nipple, giving rise to a few long radial branches – the efferent ducts – which in turn give rise to a moderate number of branches which terminate in small single alveoli with a double row of cubical epithelium. However these alveoli are sharply distinguishable from the alveoli of the functioning gland. They are very small, they have hardly any lumen and no secretion is present in them. They are blind epithelial processes.

As we have observed, in the stage of proestrus dense proliferative nodules of epithelial cells, sharply demarcated from the connective tissue stroma, are formed at the ends and along the course of the smallest efferent ducts (Fig. 1). The epithelium of the small ducts and the alveoli proliferates and the alveoli increase in size. In the subsequent phases – estrus and metestrus – part of the proliferating nodules are converted into alveoli. In the course of diestrus the greater part of the alveoli undergo regression. The remaining alveoli and the cells of which they are composed, become greatly diminished in size. These processes take place without interruption in the mammary gland of sexually mature nonpregnant mice in accordance with the cyclic changes in the ovary. It must be pointed out that the degree of the changes described during the transition from one phase of the cycle to the next varies in different individuals and sometimes it can only be made out with difficulty.

The epithelial cells, and especially their nuclei, in the small ducts and terminal portions also undergo uninterrupted changes, showing in the majority of the phases of the sexual cycle an extreme polymorphism and very great variations in size. All intermediate stages can be found, from large, spherical, lightly stained nuclei to small, dense, darkly stained nuclei in the form of blacksmiths' nails or clumps of various shapes.

The terminal segments in the gland of the nonpregnant mouse are so few in number that in order to count the mitotic activity it is essential to examine a large number of sections. In this connection it cannot be excluded that in some cases small efferent ducts, cut across obliquely or tangentially, might have been taken by mistake for a terminal segment.

The process of proliferation of epithelial cells is balanced by the process of their physiological death. Death of surplus epithelial cells follows two paths in accordance with the differing situation of the epithelium: the first path is desquamation of epithelial cells with pyknotic nuclei into the lumen of the efferent ducts and alveoli (Fig. 2), the second path is swelling of the epithelial cells of the proliferative nodules, followed by their lysis. The first path is quicker, occurs during diestrus and proceeds without the intervention of leucocytes. The second path is of longer duration, lasting throughout metestrus, diestrus and even proestrus. It may be that this process does not come to an end in the course of one cycle and extends into the next. However such a suggestion requires closer definition. It is of interest to point out that the second path of destruction of epithelial cells takes place only with the obligatory participation of granulocytic leucocytes and lymphocytes, which infiltrate the resolving epithelial nodules (Fig. 3).

We shall give a short account of the state of the mammary gland, mainly so far as the epithelium of its terminal segments is concerned, in the various stages of the cycle.

Proestrus. At the ends of the small ducts of the gland and along their extent, proliferative nodules arise, in which mitotic figures are often found (see Fig. 1). The nodules are sharply demarcated from the connective tissue stroma. The nuclei of the epithelial cells are polymorphous. There are few dense, darkly staining nuclei and more large and lightly staining nuclei. Nuclei in the form of blacksmiths' nails are encountered rarely. Desquamation of cells into the lumen of the ducts is insignificant. There are few leucocytes in the connective

The Mitotic Activity of the Epithelium of the Mammary Glands of Mice in Various Stages of the Sexual Cycle

Stage of cycle	No. of mitoses	Mitotic coefficient (per 1000)	Early prophase		Prophase		Metaphase		Anaphase		Telophase		Late Telophase	
			absolute	in %	absolute	in %	absolute	in %	absolute	in %	absolute	in %	absolute	in %
Proestrus	369	6,8	40	10,84	92	24,93	91	24,66	9	2,43	85	23,03	52	14,02
Estrus	422	7,0	39	9,24	101	23,93	69	16,35	7	1,66	96	22,75	110	25,07
Metestrus	251	4,2	19	7,57	73	25,10	42	16,73	3	1,39	55	22,75	59	23,51
Diestrus	69	1,2	6	8,69	25	36,23	7	10,14	1	1,45	13	18,84	17	24,65

tissue stroma. Collections of large epithelial cells with lightly stained nuclei, not so sharply demarcated from the connective tissue stroma may be found. The chromatin in the cell nuclei is concentrated in granules and clumps, irregular in shape. Between the cells are many granulocytes and lymphocytes. The impression is created that some of these collections are not connected with the general epithelial framework of the gland. We believe that these are old, disintegrating nodules of epithelium.

Estrus. Part of the proliferative nodules have acquired a lumen and are forming alveoli. The nuclei of the epithelial cells of the terminal segments are polymorphous and there are many dense, darkly staining nuclei, part of which is shaped like a blacksmith's nail. Desquamation of cells into the lumen of the ducts is insignificant. Leucocytes are encountered rarely in the stroma.

Metestrus. The proliferative nodules consist of cells with round, not so clearly demarcated nuclei. Between the epithelial cells are many leucocytes. In the wall of the alveoli the cell nuclei are round and lightly stained. Dense, darkly stained nuclei are rarely found. Desquamation of cells into the lumen of the ducts is almost unnoticeable. The connective tissue stroma of the gland is infiltrated by leucocytes to a greater extent than in the other stages of the cycle.

Diestrus. The number and size of the alveoli are diminished. The nuclei of the epithelial cells of the terminal portions are polymorphous and variable in size. Many dense, darkly staining nuclei shaped like blacksmiths' nails and atrophic, pyknotic nuclei are present. Desquamation of epithelial cells into the lumen of the ducts and alveoli is observed (Fig. 2). There are fewer leucocytes in the stroma than in metestrus, but more than in the phases of estrus and proestrus.

Determination of the mitotic activity (see Table) shows that it does not stay constant, but varies throughout the whole cycle. The lowest degree of mitotic activity is observed in the period of diestrus (1.2 mitoses per thousand cells on the average); it rises sharply during proestrus (on the average 6.8 mitoses per thousand cells) and remains high throughout estrus (7.0 mitoses per thousand cells on the average).

The difference in the mean values of the mitotic activity at the various stages are statistically significant ($P=0.001$). The exception is the difference between the mitotic activity in the periods of proestrus and estrus. Here they are not statistically significant. The smallest individual variations were observed in estrus (from 5 to 9 mitoses per thousand cells). In the remaining phases these variations were significant. The highest value of the mitotic activity was observed in certain animals in proestrus (15.2 mitoses per thousand cells). There are therefore grounds for believing that at a certain period of proestrus the mitotic activity is maximum on account of the intensive division of the cells of the proliferative nodules. In diestrus the mitotic activity, although it varies considerably in individual animals (from 0.3 to 3.5 mitoses per thousand cells), nevertheless remains low in comparison with the phases of estrus and proestrus. Evidently at the beginning and end of diestrus the mitotic activity is not the same, and this may account for its individual variations in animals killed at the various stages of this period. The low figures for the mitotic activity in diestrus enable us to deny categorically the existence of a second significant rise in mitotic activity as described in this period by Bullough.

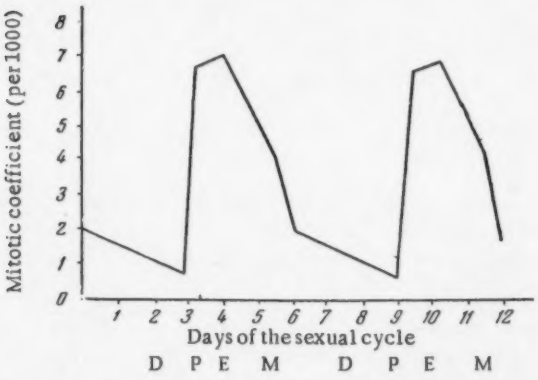

Fig. 4. The mitotic activity of the epithelium of the mammary gland of the mouse in different stages of the sexual cycle.

Comparison of the percentage relationships of the individual phases of mitosis of the epithelial cells of the mammary gland at different periods of the sexual cycle shows that in proestrus, when mitotic activity rises sharply, the percentage of metaphases increases. The mitotic activity is 8% higher than in the period of estrus; however this difference is not statistically significant.

The curve expressing the changes in the mitotic activity of the epithelium of the mammary gland (Fig. 4). shows that increased mitotic activity coincides with the secretion of estrogenic hormones from the ovaries into the blood. In diestrus, when estrogenic hormones are not secreted by the ovaries, the mitotic activity of the epithelium of the mammary glands is minimal.

In nonpregnant mice, in physiological conditions the appearance of estrogenic hormones in the blood leads to stimulation of cell division. However this still does not prove a direct action of estrogenic hormones on the epithelium of the mammary gland. Ferguson [4] showed in castrated and hypophysectomized female mice that the estrogenic hormones alone, without prolactin and pituitary growth hormone, do not have any stimulating effect on the mammary gland.

SUMMARY

The mitotic activity of epithelium of the end portions of the mammary glands was studied during various stages of sexual cycle in nonlinear white mice. The rise of mitotic activity corresponds to the maximal effect of estrogenic hormones in time. The mitotic activity is maximal in estrus and proestrus and is equal, on the average, correspondingly to 6.8 and 7.0 mitoses per thousand epithelial cells. In diestrus the mitotic activity is minimal and equals, on the average, 1.2 mitoses per thousand cells.

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ACTIVATION OF THE DIVISION AND GROWTH OF CELLS DURING REGENERATION

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One of the characteristic features of the regenerative process is the proliferation and hypertrophy of cells. Cell division takes place both in organs with high mitotic activity and in several organs whose cells have lost their ability to undergo mitosis in the process of ontogenesis. The mechanism of activation of division and growth of cells during regeneration has been little studied until now. We have shown that the occurrence of cell division during regeneration is connected with activation of the cells by protein products of disintegration of tissues. Injection of protein into the animal caused hypertrophy and division of cells in organs in which under physiological conditions mitoses are absent [1, 2]. Activation of growth and cell division by protein or by products of its incomplete decomposition is effected via the nuclein metabolism of the cell [3]. These findings were obtained mainly in studies of regeneration of the liver.

It appeared likely that the same mechanism lies at the basis of regeneration of other organs. In this connection experiments were carried out in which activation of division and growth of cells was studied during regeneration of the salivary glands.

EXPERIMENTAL METHOD

Experiments were carried out on white mice from 2½ to 3 months old. In the first series of experiments about two thirds of the right submandibular salivary gland was extirpated from the animals. As a control, mice

TABLE 1

Changes in the Mitotic Activity and Dimensions of Cells of the Salivary Gland during Regeneration

Group of experiments	No. of animals	No. of mitoses M + m and coefficient K	Probability p	Area in μ^2 (M ± m)			
				cell	t	nucleus	t
Control	4	2,2±0,5; 1,5	—	91,1±2,2	—	17,5±0,54	—
Regenerating gland	6	11,6±2,0; 1,1	0,002	104,0±4,0	2,85	19,3±0,37	9,0
Undamaged gland	6	6,6±1,1; 2,0	0,006	102,9±2,7	3,45	22,1±0,48	6,6
Control	5	0; —	—	100,5±1,7	—	18,0±0,33	—
Regenerating gland	9	2,6±0,4; 1,5	0,000	135,5±2,7	10,8	23,5±0,42	10,0
Undamaged gland	9	2,?±0,4; 1,3	0,000	124,8±2,2	10,0	22,2±0,54	6,0

from the same litter were used, which were kept under identical conditions but not subjected to the operation. The animals were killed at the same time, 3 days after operation. Histological examination of the regenerating (top) and undamaged (bottom) salivary glands was performed. The mitotic activity was determined by counting the number of dividing cells in a constant area (1.65 mm^2) and by calculation of the phase coefficient (the ratio of the first two phases of mitosis and the last two phases). The areas of the transverse section of the cells and of their nuclei were measured by drawing the projections of the cells and by planimetry of these drawings. In both the operated and the undamaged glands 100 cells were measured in each (50 serous and 50 mucous). In each control and each experimental group of animals measurements were made on the glands of 2-3 mice. The mean values of the dimensions of the cells, given in Tables 1-4, are thus based on measurements of 200-300 cells. By analysis of the distribution curves of the measurements of the serous and mucous cells we obtained identical results, and so to simplify the tables and the figures we included in them the combined values for both types of cell. Besides determination of the mitotic activity and the dimensions of the cells, in each experiment determinations

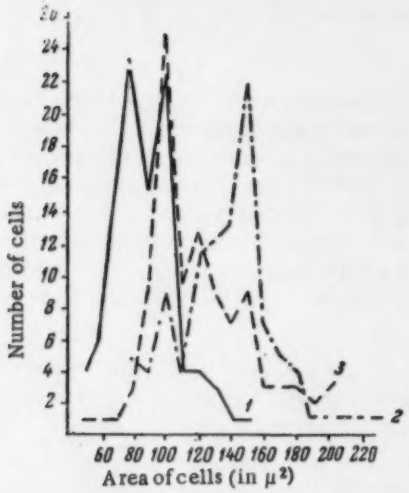

Fig. 1. Distribution curves of measurements of the cells of the salivary glands.

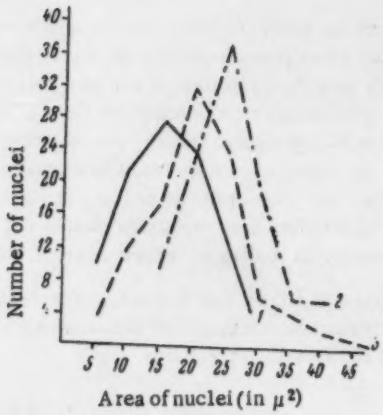

Fig. 2. Distribution curves of the dimensions of the nuclei. Symbols as in Fig. 1.

were made of the ribonucleic (RNA) and desoxyribonucleic acid (DNA) contents of the cell. The material was fixed in Helly's mixture. RNA was detected by staining with methyl green and pyronin by Brachet's method and DNA by the Feulgen reaction.

In the second and third series of experiments, the changes in mitotic activity, dimensions of the cells and DNA and RNA content of the cells of the salivary glands were investigated after injection of protein extracts of various organs. The organ was chopped up finely, extracted with saline for 1-1½ hours in the cold and centrifuged. The extract (0.5-1% of protein) was injected intraperitoneally into white mice in doses of 0.2 cm^3 for 3 days. The material was fixed 3 days after the last injection. In experiments on chick embryos the extract (0.05 cm^3) was injected once only.

EXPERIMENTAL RESULTS

In the salivary gland of the mouse only single dividing cells are found. In many cases in an area of 1.65 mm^2 it is generally speaking impossible to observe mitoses. During regeneration the mitotic activity of the salivary gland increases 3-5 times (Table 1). At the same time an increase in the dimensions of the cells and of their nuclei is observed. Analysis of the curves of distribution (Fig. 1) shows that the increase in the mean dimensions of the cells is connected with a reduction in the number of small cells, with increase in the number of large cells and with the appearance of very large glandular cells which are not met in the control animals.

The number of cells in the classes of average dimensions underwent comparatively little change in the series of experiments. An increase in the dimensions of the nuclei and a clear shift of the distribution curve to the right were also observed (Fig. 2).

Hypertrophy of cells during regeneration of the salivary glands was expressed quite clearly, although to a lesser degree than in regenerating liver. As in other organs, regeneration of the salivary glands was accompanied by a marked increase in the RNA and DNA content of the cells. On the 3rd day after the trauma a marked increase in the intensity of staining of the cytoplasm with pyronin and of the nuclei with Schiff's reagent was observed in the regenerating lobe.

If activation of division and growth of cells during regeneration is connected with a humoral mechanism, it would be expected that it would extend also to the undamaged gland on the opposite side. As can be seen from Table 1 and Figures 1 and 2, the same increase in mitotic activity and in the dimensions of the cells and of their nuclei is observed in the undamaged gland as in the regenerating gland. The intensity of these changes in the undamaged organ is only a little less than in the salivary gland subjected to operation. In the undamaged gland the DNA and RNA content of the cells is also increased. Thus the increase in mitotic activity, hypertrophy of the cells and increase in the DNA and RNA content of the cells extends not only to the regenerating organ but also to the intact analagous organ on the opposite side. The findings which we obtained from a study of regeneration of the liver suggest that activation of the division and growth of the cells of a regenerating organ is connected with the activity of protein products of decomposition of tissue as a result of the trauma. In this connection we carried out a second series of experiments in which healthy white mice were injected with a salivary gland extract. On the 3rd day after a third injection of the extract, the intact salivary glands showed an obvious increase in mitotic activity and in the dimensions of the cells and of their nuclei (Table 2), which was even more sharply expressed than during regeneration (Fig. 3).

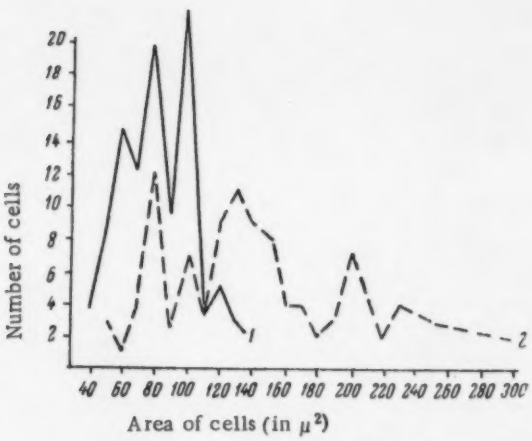

Fig. 3. Distribution curves of the dimensions of the cells of the salivary glands.

Injection of extract, like regeneration, caused a marked increase in the intensity of the reaction to RNA and DNA. After injection of the extract of

Table 2

Changes In Mitotic Activity and the Dimensions of the Cells of the Salivary Gland after Injection of an Extract of this Organ

Group of experiments	No. of animals	No. of mitoses M + m and coefficient K		Probability P	Area μ^2 (M = m)			
					cell	t	nucleus	t
Control	6	0.6±0.2;	—	—	100.8±2.6	—	19.5±0.41	—
Injection of extract	8	5.1±0.4;	3.3	0.000	162.7±4.1	12.8	24.3±0.38	8.6
Control	8	0.1±0.1;	—	—	79.2±1.5	—	19.2±0.33	—
Injection of extract	6	4.1±1.1;	1.0	0.004	126.5±3.3	13.8	26.0±0.48	11.7

salivary gland the mitotic activity in this organ increased more than tenfold. Thus the injection of a protein extract literally reproduces the changes which are observed during regeneration. The identity of these changes suggests that the activation of division and growth of cells observed during regeneration is connected with the activity of protein products of decomposition of tissues as a result of trauma. If the proteins are precipitated (by heating to 60-70°C) the organ extracts lose their ability to stimulate mitotic activity. Processes of proteolysis and the accumulation in the focus of trauma of protein and the products of its incomplete decomposition are, from our point of view, one of the essential factors stimulating the hyperplasia and hypertrophy of the regenerating organ. Since the changes in the RNA and DNA content of the cell precede the stimulation of mitoses and hypertrophy of the cell [3], it can be suggested that activation of cells by protein products of tissue decomposition is effected through the nuclein metabolism of the cell. Data on the role of RNA in the processes of protein syntheses [4, 6-10 and others] and on the importance of nuclein metabolism in cell division [5, 8 and others] make this a likely hypotheses.

Experiments in which various extracts were injected (Table 3) demonstrate the organ specificity of the activating effect of protein on cell division. As can be seen in Table 3, injection of an extract of salivary gland stimulates mitotic activity in this organ only and does not change it appreciably in the epithelium of the cornea

TABLE 3

The Influence of Extracts of Various Organs on Mitotic Activity

Group of experiments	No. of animals	No. of mitoses M ± m and coefficient K							
		salivary gland		P	cornea	P	Intestine		P
Control	8	0.1±0.1;	—	—	120±17; 1.0	—	—		—
Extract of salivary gland	6	4.1±1.1;	1.0	0.000	113±12; 1.1	0.922	—		—
Control	6	0.6±0.2;	—	—	160±15; 1.1	—	311±19;	1.2	—
Extract of salivary gland	8	5.1±0.4;	3.3	0.000	132±15; 1.1	0.218	281±11;	1.2	0.696
Control	7	0.3±0.1;	—	—	62±15; 1.7	—	302±10;	1.7	—
Extract of intestine	7	0.1±0.1;	—	0.187	63±19; 1.8	0.922	339+19;	1.5	0.626

or the intestine. At the same time injection of an extract of intestine does not change the mitotic activity in the salivary gland.*

Thus in this series of experiments an organ specificity was apparent in the action of the protein, which we might have surmised on the basis of our experiments on regeneration of the liver[2].

The results of the experiments in which various extracts were injected impelled us to ascertain whether the reaction to injection of protein is preserved in the embryonic period, in conditions when the processes of cell destruction are relatively feebly expressed. An investigation in this direction appeared to be of particular inter-

* Despite the large number of experiments in which the effect of an intestinal extract on the mitotic activity of the epithelium of the duodenum was investigated, we did not obtain any clear results. In the majority of the experiments the mitotic activity did not change and only in individual experiments was it increased to an insignificant degree. Evidently this is related to the high mitotic activity and the intensive mortality of cells occurring in this organ. Probably it is for this very reason that the action of excessive protein does not give here the same effect as is observed in the salivary gland and liver.

est in connection with the findings of a number of workers [7, 11] who demonstrated that injection of protein into an embryo caused an increase in the weight of that particular organ. It seemed likely that these changes are based on the same mechanism of activation of growth and division of cells with which the process of regeneration is connected. We therefore undertook a third series of experiments – on 7-day old chick embryos. Into the vascular field of the embryo was injected 0.05 cm^3 of an extract of the liver of a sexually immature chick of the Leghorn breed. The embryos were fixed 2-3 days after the injection. The ratio of the weight of the liver to the weight of the D embryo, the dimensions of the liver cells and the mitotic activity in the liver were investigated (mitotic activity in an area of 3.3 mm^2).

TABLE 4

The Effect of a Liver Extract on the Division and Growth of the Liver Cells of the Embryo

Group of embryos	D	Area, in μ^2 (M ± m)				No. of mitoses and coefficient K
		cell	t	nucleus	t	
Control	0.0155	88.2±2.5	—	24±0.67	—	143; 1.6
»	0.0101	101.6±2.9	—	24±0.67	—	132; 1.7
Liver extract	0.0223	137.2±4.9	6.9	29±0.87	4.0	201; 3.0
as above	0.0189	131.2±3.5	7.1	28±0.68	3.3	212; 2.9
Control	0.0139	70.4±2.8	—	16.2±0.47	—	71; 1.8
»	0.0112	73.6±1.5	—	16.5±0.51	—	59; 2.2
Liver extract	0.0163	121.8±6.1	7.2	23.2±0.53	8.5	168; 2.5
as above	0.0151	93.6±4.6	3.8	21.4±0.58	5.8	120; 2.5
» »	0.0148	101.4±3.8	5.9	20.7±0.42	5.6	118; 2.2

As can be seen in Table 4, in which are shown the results of two groups of experiments, injection of liver extract causes a relative increase in the weight of the liver. These changes are associated with hypertrophy of the cells and an increase in mitotic activity. Thus even in early stages of development injection of protein causes growth and division of cells. This reaction is organ specific in character. In experiments in which an extract of the spleen was injected we were unable to observe any changes in the mitotic activity or dimensions of the liver cells.

The experiments carried out on chick embryos suggest that activation of mitoses and of growth of cells by protein products of tissue destruction is a phylogenetically primitive reaction of cells to trauma. This is the simplest reaction of the protoplasm, bringing about the stability and preservation of the organism in the midst of the harmful influences of the external environment, and it was evidently established long before the formation of the complex neurohumoral regulation of the growth and division of cells. Death of some cells (or of parts of them) led to the growth and proliferation of others. The experiments of Weisz [15] on the conjoined infusoria (Stentor) provide weighty arguments in support of this point of view. The reaction of cells to the action of protein products of tissue destruction is also preserved in the higher animals as the simplest means of regulation of reparative and, evidently, of physiological [12-14] regeneration. Liberation of protein and the products of its incomplete decomposition lead under these conditions to alterations in the nuclein metabolism and, hence, to growth and division of the cell. The observations described thus suggest that the development of mitoses and of hypertrophy of the cell during regeneration is connected with activation of the cells by protein products of tissue decomposition resulting from trauma.

SUMMARY

An increase of mitotic activity, the size of the cells and their nuclei and the increase of content of RNA and DNA was noted in regeneration of salivary glands in mice. Analogous changes were noted in uninjured gland on the contralateral side. Administration of an extract of salivary gland caused the rise of mitotic activity, an increased content of nucleic acids in the cells and hypertrophy of the cells. Injection of liver extract into the

check's embryo brought about an increase of the relative weight of the liver, the rise of mitotic activity and cellular hypertrophy. A conclusion was drawn that protein and the products of its disintegration possesses an organospecific effect on the growth and mitosis of cells.

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CHANGES IN MITOTIC ACTIVITY IN RATS IN RELATION TO THE TIME OF DAY OR NIGHT

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On the basis of reports in the literature [8-10, 12-19] it can be taken as established that there is a daily rhythm of mitotic activity in the body. This leads to the thought that in the normal animal there are conditions which regulate the process of mitotic division. In recent years data has been obtained in this direction by G. S. Strelin and his co-workers [7], L. A. Alov and his co-workers [1, 2], S. Ia. Zalkind [3] and others. It is natural to suggest that disturbance of these conditions may lead to abnormality of the daily rhythm of cell division.

The question arises whether these changes in the daily rhythm of mitotic activity in the body arise during various pathological states, and in particular in malignant growth. Very few investigations have been devoted to this problem [1, 8, 11, 20]. The scanty reports in the literature on the character and intensity of the changes in the daily rhythm of mitotic activity do not enable any definite opinion to be formed on the laws affecting this phenomenon.

In this connection it appeared desirable to carry out research in order to shed light on the following problems: 1) What is the daily periodicity of the processes of cellular proliferation in the different meristems* of the normal animal? 2) Is the mitotic activity changed in these meristems in the course of day and night when a focus of malignant growth is present in the body? 3) Is there a daily rhythm of cell division in the tumor itself?

EXPERIMENTAL METHOD

We carried out experiments on white rats (males). Altogether 80 animals were used in a control series and 64 rats in the experimental series, weighing from 90 to 110 g. The animals of the experimental series were inoculated with sarcoma M-1. The rats were killed by decapitation at intervals of 3 hours, i. e. 8 times in the course of 24 hours. The material was fixed in Zenker's solution; staining was with hematoxylin by Caracci's method. The mitotic activity was assessed by calculation of the mitotic coefficient – the ratio between the number of dividing cells and the total number. The whole of the results were treated statistically by the Fisher-Student method.

EXPERIMENTAL RESULTS

The purpose of the first control series of experiments was to establish the character of the daily periodicity of cellular proliferation in the cornea of the eye, the epidermis of the skin and the crypts of the small intestine in normal rats. In Fig. 1 the changes in the mitotic coefficient in the course of the 24 hours in the three structures indicated are expressed graphically.

It follows from the numerical data for the mitotic coefficients obtained for the corneal epithelium of normal rats that, starting at 12 noon the number of mitoses in this tissue gradually falls. The minimum number

* As in original.

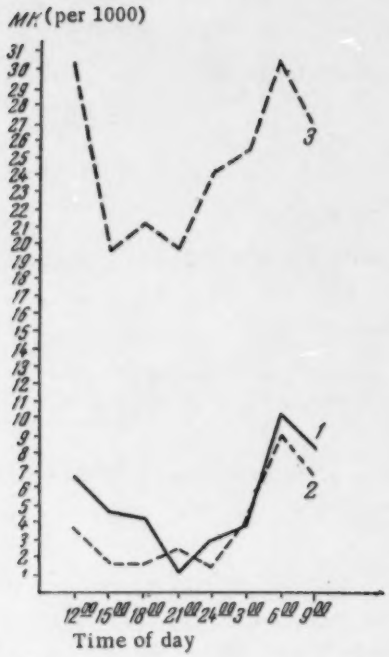

Fig. 1. The mitotic coefficient (MK) in different meristems of normal rats in the course of 24 hours. 1) corneal epithelium; 2) epidermis of the skin; 3) epithelium of the small intestine.

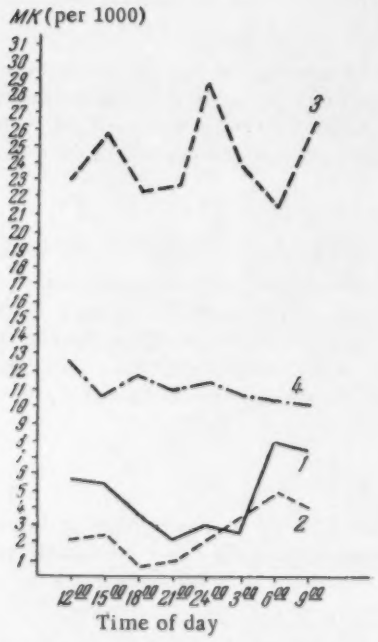

Fig. 2. The mitotic coefficient in different meristems of rats inoculated with a tumor, in the course of 24 hours. 1) corneal epithelium; 2) epidermis of the skin; 3) epithelium of the small intestine; 4) M-1 sarcoma.

of dividing cells is found at 9 p.m. The fall occurring in the interval between 6 p.m. and 9 p.m. is statistically significant (P=0.002). Next the number of dividing cells gradually increases and reaches a maximum value at 6 a.m. The increase in mitotic activity between 3 a.m. and 6 a.m. is statistically significant (P=0.005). Although the level of mitotic activity in the corneal epithelium is lower at 9 a.m. than at 6 a.m. (P=0.05), it remains relatively high and at approximately the same level as at 12 noon.

The results described demonstrate that in the corneal epithelium of normal rats there are daily changes in mitotic activity, and the curve of these changes is seen to be clearly unimodal in character.

The changes in the mean values of the mitotic coefficients in the epidermis of the skin at various times of day and night also demonstrate the presence in this meristem of a daily rhythm of cellular proliferation, and the curve of the changes in mitotic activity is unimodal in character. Starting at 12 noon a fall in the mitotic activity occurs (P=0.01), the subsequent variations of which are not statistically significant. The minimum number of divisions is observed at 12 midnight, after which a sharp rise takes place in mitotic activity, reaching a maximum value at 6 a.m. (in the interval between 3 a.m. and 6 a.m. P=0.01). The fall in the value of the mitotic coefficient to 9 a.m. and later is insignificant, and hence the period from 3 a.m. to 12 noon may be regarded as a time of relatively high mitotic activity in the epidermis of the skin.

As in the first two cases (cornea of the eye, epidermis of the skin), starting at 12 noon the value of the mitotic coefficient for the epithelium of the crypts of the small intestine falls sharply. By 3 p.m. the mitotic activity is already at a minimum and in the interval between 3 p.m. and 9 p.m. it remains practically at the same level. At 12 midnight and later the level of mitotic activity gradually rises; the maximum number of dividing cells is also found at 6 a.m. As also in the case of the epidermis of the skin, the period from 12 midnight to 12 noon must be regarded as a time of relatively high mitotic activity.

Thus in all the meristems of normal rats examined there exists during the course of the 24 hours a daily rhythm of cellular proliferation, and the curve of the changes in mitotic activity is unimodal in character. The mitotic activity is known to characterize the degree of renewal of cells in the particular tissue, i. e. the level of its physiological regeneration. The results obtained enable the conclusion to be made that the level of physiological regeneration is lower in the epidermis of the skin by comparison with the corneal epithelium and, more especially, with the epithelium

of the crypts of the small intestine, where the mitotic activity is 3-4 times higher than in the cornea.

In all the tissues studied we determined not only the number of dividing cells but also the phases of mitosis. We were unable to establish any essential changes in the relationships between the phases of mitosis at the different times in the 24 hours.

The purpose of the second series of experiments was to elucidate the problem of the character of the possible changes in the daily periodicity of cellular proliferation in the different meristems of the animal affected with a focus of malignant growth. The experimental rats were killed on the 15th day after inoculation of a M-1 sarcoma into the thigh muscles. At the moment of death, the weight of the tumors in the different animals varied between 10 and 40 g.

Comparison of cellular proliferation in the cornea of the control and experimental animals demonstrates the identity of the character of the daily rhythm of mitotic activity in normal animals and in those affected by a malignant growth (Fig. 2). The smallest number of divisions, like in the normal animal, is found at 9 p.m., and the maximum mitotic activity is observed at 6 a.m. However it should be pointed out that the extent of the variations of the mean values of the mitotic coefficients in the cornea is shown to a lesser degree in the experimental than in the control animals.

The same can be stated concerning comparison of the daily changes in mitotic activity in the epidermis of the skin of normal and experimental animals: in their character these changes are of the same type. The daily variations in the mitotic activity of the crypts of the small intestine are shown somewhat differently in the experimental animals. In animals inoculated with tumors the fall in the value of the mitotic coefficient in the crypts of the small intestine begins at 3 p.m. and continues at approximately the same level until 9 p.m. The maximum number of divisions is observed at 12 midnight, not at 6 a.m. as in normal animals. In the interval from 6 p.m. to 12 midnight the difference in the values of the mitotic coefficient is statistically significant, although the sharp fall in the level of mitotic activity at 6 a.m. is not significant in character, as is also the case with its increase which commences at 9 a. m. Consequently in an animal affected by a malignant growth the variations in the mitotic activity in the crypts of the small intestine demonstrate a disturbance of the character of the daily rhythm of cell division. In addition the maximum and minimum values of the mitotic coefficients in the experimental rats are smaller than in the controls.

How does the value of the mitotic coefficient change in the course of the 24 hours in the tumor itself?

Analysis of the available data leads us to the conclusion that a daily rhythm of cell division is absent from the transplanted M-1 sarcoma. The value of the mean mitotic coefficients is practically unchanged at different hours of the day or night; under these conditions it is slightly higher than the value of the maximum mitotic activity of the corneal epithelium and of the epidermis of the skin in normal rats, but significantly lower than that in the epithelium of the small intestine.

Thus the absence of a daily rhythm of mitotic activity in the tumor may be evidence of a definite peculiarity of the process of cellular proliferation.

The results which we obtained confirm the observations made by several authors [9-11, 16, 19] on the presence of a daily periodicity in cellular proliferation. The curve of mitotic activity has a unimodal character. At the same time our results do not agree with the findings of other workers [12, 15] who find that the curve of the daily rhythm of mitotic cell division is bimodal. This is evidently accounted for by the fact that in Bullough's [12] work a method was used in which material was repeatedly taken from the same animal.

From the data in the literature there is some doubt about the problem of the cynchrony of the daily changes in mitotic activity in the different meristems of the same animal. According to some authors [10] the daily changes in the different meristems are not of the same type, whereas other workers [12] maintain the opposite view. This divergence of opinion may be explained by the fact that each of these authors cited used different meristems of the animal for comparison.

The problem of the synchrony of the changes in mitotic activity in different tissues is of great importance in solution of the question of the degree of specificity of the factors regulating cell division in different meristems of the animal. The experimental findings which we obtained demonstrate that the daily changes in cell

division in the cornea, the epidermis of the skin and the epithelium of the crypts of the small intestine in normal rats belong to the same type.

So far as the changes in the daily periodicity of cellular proliferation in rats affected by an inoculated M-1 sarcoma are concerned, our findings suggest some disturbance with the rhythms established in normal animals. We point out the reduction in the range of variations of the mean values of the mitotic coefficients in the course of the 24 hours and the displacement of the maximum number of cell divisions in the crypts of the small intestine from daytime to nighttime.

The question of the effect of a malignant growth on the changes in the daily periodicity of cellular proliferation may be finally settled by investigations on spontaneous or indiced tumors.

SUMMARY

Mitotic activity in the corneal epithelium, cutaneous epidermis and cryptic epithelium of the small intestine of normal rats is characterized by a distinct 24-hour rhythm.

In all of these tissues the maximum number of cellular divisions was observed in the morning and the minimum in the evening. In rats with sarcoma M-1 (transplanted subcutaneously) no distinct changes were discovered in the 24-hour rhythm of the cellular division of the same tissues. The 24-hour rhythm of the cellular division is absent in the transplantable M-1 sarcoma.

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METHODS

A PLASTIC SUPERCONDENSER (3D)

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Statement of the problem. Investigations on biogranules carried out in our Institute required improvements in the technic of microscopic observations. Our aim was to increase the resolving power of the microscope and to obtain undistorted plastic pictures of unfixed, unstained, living biological material.

According to Abbé's teaching the microscopic picture in stained preparations is due to absorption, and in unstained preparations to diffraction. In the latter case the rays of light, after passing through the fine structures of the object, undergo precisely the same changes as on passing through an optic grating. Light falling on the object is dispersed into the so-called diffraction beams. The center one of these diffraction beams (0) passes through the optic media of the object without changing its initial direction. The lateral beams are inclined to the optic axis at an angle α, whose value is greater the higher the serial number of the beam. (Fig. 1).

The magnitude of the angle α can be determined by considering the wavelength of the light (λ), the width of the tissue elements or the intervals of the grating (d) and, finally, the refractive index of the medium occupying the space between the object and the objective (n). For the first beam, this magnitude is determined by the expression:

$$\sin \alpha = \frac{\lambda}{n \cdot d}.$$

In accordance with the theoretical and experimental investigations of Abbé, in order to obtain an image similar within limits to the object it is essential that besides the median diffraction beam at least one other diffraction beam of light should fall on the microscope (Fig. 1). In a case where the objective is situated very close to the object (for high magnification), this can take place at an angle α=70-72°

(sin α 72°=0.95).

The smallest distinguishable width of the spaces of the grating or the finest unit of structure of tissue (d) in microscopic examination corresponds to the limit of the resolving power of the microscope, i. e. is equal to the smallest distance between two points on the object from which it is possible to obtain separate points on the image

$$d = \frac{\lambda}{n \sin \alpha} = \frac{\lambda}{A}$$

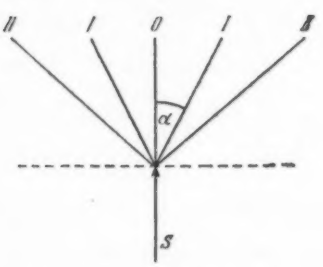

Fig. 1. Diagram showing the arrangement of the diffraction beams during passage of the light through the fine structures of an object (according to Abbé). For description see text.

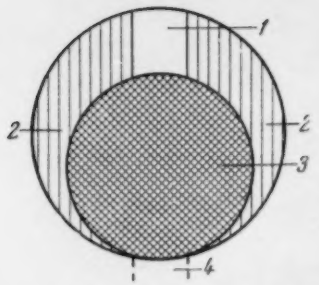

Fig. 2. Arrangement of the separate fields of illumination in the plane of the object in the method of combined illumination by Zselionka and Kiss. For description see text.

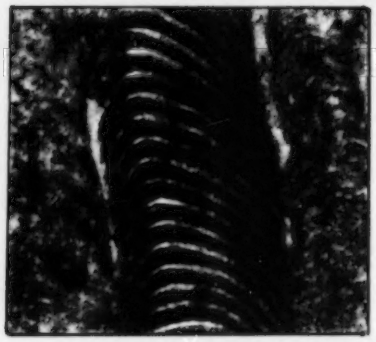

Fig. 3. An area of transversely striated muscle from a human cadaver exhumed 492 days after death (preparation of Prof. Ekresh).

Microphotograph produced by the use of the supercondenser (3D).

where A=n sin α; A is the so-called numerical aperture of the system.

If d is smaller than this value, then beam I does not fall on the microscope and no image is formed. The field of vision becomes uniformly illuminated. Thus the resolving power of the microscope (d) is greater the smaller λ and the greater A.

The wavelength of the greenish yellow light which acts most strongly on the eyes is (λ)=0.55 μ.

When a dry lens system is in use, n=1, since the light enters the objective from air. Consequently in this case A=1·sin 72°=1·0.95.

The resolving power of the microscope when working with a dry lens system is thus:

$$d = \frac{0{,}55}{0{,}95} = 0{,}58\ \mu.$$

For an oil immersion objective this value is:

$$d = \frac{0{,}55}{1{,}51 \cdot 0{,}95} = \frac{0{,}55}{1{,}4} = 0{,}4\ \mu.$$

With lateral illumination:

$$d = \frac{\lambda}{2A},$$

i. e.

$$d = \frac{0{,}55}{2 \cdot 0{,}95} = \frac{0{,}55}{1{,}9} = 0{,}3\ \mu \text{ (for dry objectives)}.$$

$$d = \frac{0{,}55}{2{,}8} = 0{,}2\ \mu \text{ (for oil immersion)}.$$

These values are the limits, determined on theoretical grounds, of the resolving power of the microscope. Further increase in this power is possible only by means of reduction of the wavelength (λ) (Abbé).

If d is equal to 0.4 μ, then in a microscope image of an area of the object 10 μ in length, 25 distinct points can be made out; if d=0.2 μ, then in this distance of 10 μ 50 separate points can be distinguished optically.

By the use of ultraviolet light (λ=0.275 μ) the resolving power of the microscope is increased to

$$d = \frac{0{,}275}{2 \cdot 1{,}4} = 0{,}1\ \mu \qquad \text{(Köhler)}$$

In this case quartz objectives are used and the source of light is an arc lamp with cadmium electrodes.

From these considerations it is understandable that improvements in the microscopic image can be obtained not only by changes in the objective and ocular of the microscope but also by improving the illumination of the object. We chose this latter route in the construction of our supercondenser 3D, which is based on a novel combined illumination.

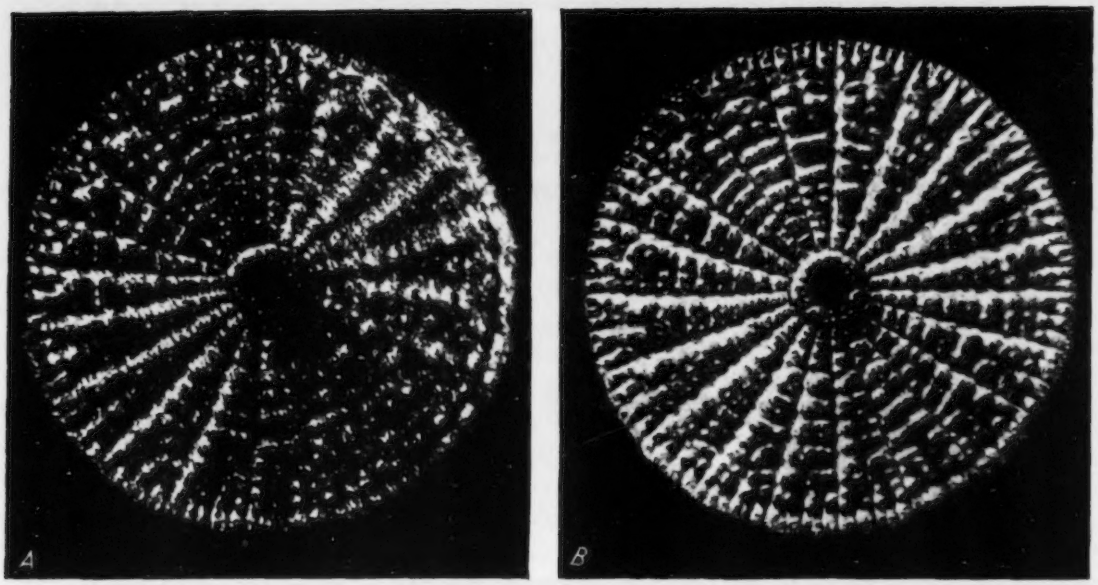

Fig. 4. A — microphotograph produced by means of a cardioid condenser; B — microphotograph produced by means of the supercondenser (3D).

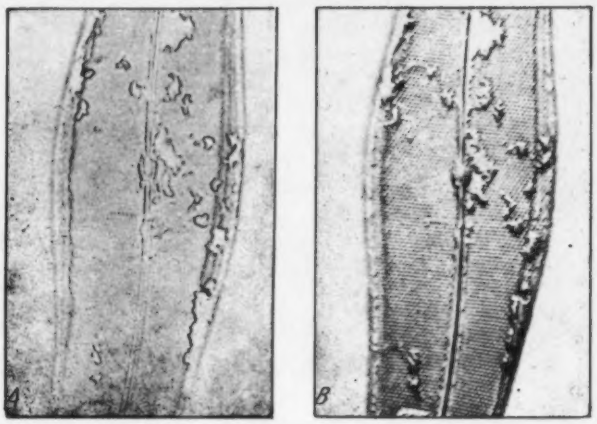

Fig. 5. A — microphotograph produced with an Abbé condenser; B — microphotograph produced with the supercondenser (3D).

Principle of the condenser 3D. The supercondenser 3D is suitable for any form of illumination by transmitted light with the exception of phase-contrast illumination. It may be used, for example, to obtain a central light field, for dark field, and in addition, for the so-called combined illumination.

Our combined illumination does away with the defects of the unilateral or circular oblique illumination in use at the present time. It is well known that with unilateral oblique illumination, adjacent points in the microscopic picture which coincide with the direction of the illuminating light, merge into a line, but a line running mainly in this direction becomes completely or almost completely imperceptible. In consequence of

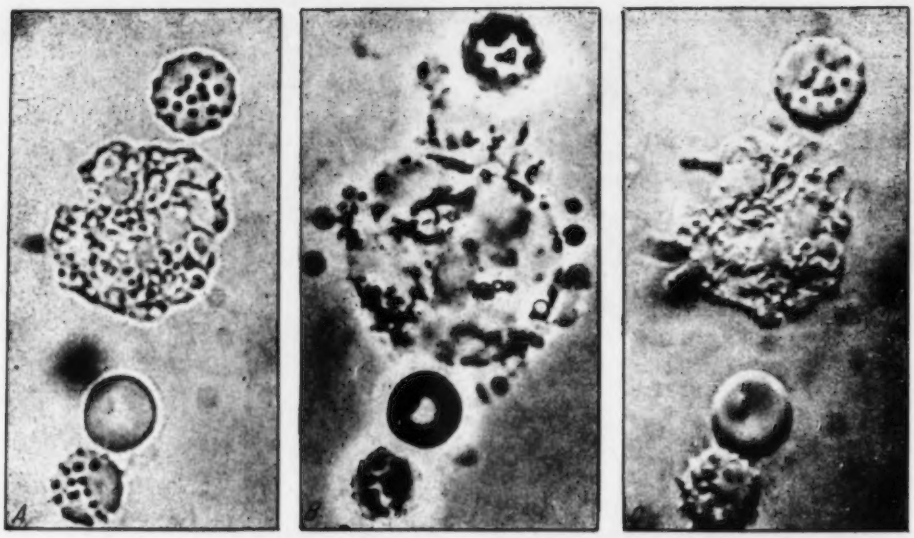

Fig. 6. Fresh, unstained human blood in a wet chamber. A – microphotograph produced by means of an Abbé condenser; B – phase contrast microscopy; C – microphotograph using the supercondenser (3D).

this,serious optical aberrations result, giving a false idea of the true structure of the object. With circular oblique illumination the optical aberrations are reduced to a minimum, but in certain cases, primarily with light from a wide aperture, the illumination of the object becomes excessive, and this leads to veiling of the image and to marked diminution of its contrast. The principle of our combined illumination is as follows: unilateral oblique illumination of considerable power is combined with light from the opposite side, leading in the same direction and creating the effect of a dark field. To this is added illumination of the field by means of crescentic beams of light of smaller intensity around it, which are partly diffuse and undergo a phase change towards a shorter wavelength of light (Fig. 2).

In Fig. 2 is shown the arrangement of the separate fields of illumination in the plane of the object with our combined method of illumination. In the 1st field is shown the action of an intense unilateral oblique illumination, whereas rays of light act in a 4th field which is arranged in opposition to the first field, to give the effect of dark-ground illumination. In a 2nd field, surrounding the 1st and 4th fields, rays of light of smaller intensity are in action, and these to some degree are filtered and become partially diffused. In the completely dark field (the 3rd field in Fig. 2) the central light (i. e. the light from a small aperture) are completely removed.

RESULTS ACHIEVED WITH OUR CONDENSER

1). Since the combined illumination produced by our method has an azimuth of 360°, optical distortions are reduced to a minimum.

2). The deep shadowing occurring with unilateral oblique illumination is weakened by the oblique light of the 4th field and also by the light of the 2nd field. In consequence of this the optically sharp borders in the individual structures of the object do not suffer any great softening of their contours.

3). The partially diffused light of the 2nd field, lower in intensity and modified towards a shorter wavelength, brings about uniformity of illumination and, in addition, increases the resolving power; on the other hand, thanks to its low intensity, it reduces the degree of illumination of the object, which increases the contrast of the image.

Thanks to the combined illumination the microscopic image of the object takes on a solid appearance; the individual details literally stand out above the surface, which creates the effect of plasticity, which is even more expressed by the use of the binocular attachment. Since our condenser provides mainly oblique rather

than central illumination, the resolving power of the system is doubled. Thanks to this we were able to approach the theoretical maximum of resolving power of the microscope more closely than by using the unilateral oblique illumination which has been used up to the present time.

The plasticity of the image is particularly suitable for investigations of the detailed structure of a surface and also in the solution of certain technological problems (Fig. 3).

The greatest external diameter of the mounting of the condenser 3D is 39.5 mm, in consequence of which it can be used in any ordinary microscope. The lenses of the condenser form an aplanatic system, whose aperture is 1.25. The supercondenser 3D has three easily replaceable frontal lenses. With one of these lenses a so-called light field similar to that with the traditional Abbé condenser may be obtained; the second frontal lens gives a plastic image; the third lens permits the effect of dark-ground illumination to be obtained.

It will be clear from this account that our condenser makes the use of the cardioid condenser unnecessary. Working with the 3D condenser is simpler than with the cardioid condenser, and the accuracy of the image obtained is in certain respects superior to that obtained by the use of the cardioid condenser (Figs. 4, 5, 6).

ADVANTAGES OF THE SUPERCONDENSER 3D

We will briefly summarize the advantages of our condenser.

1). A plastic image may be obtained, free from distortion and with a uniformly illuminated field of vision. The plasticity of the image obtained gives considerable advantages when investigating the surface of a specimen, which has so far been visible only in two dimensions even at high magnifications.

2). Increase in the resolving power of the microscope practically to the maximum attainable and, consequently, the attainment of more considerable degrees of magnification.

3). Increase in the resolving power and the use of an original method of illumination enables the production of an image by the use of achromatic objectives of a quality which is obtainable with other condenser systems only by the use of the much more expensive apochromatic objectives.

4). The construction of our condenser enables a perfect dark field to be obtained by means of simple manipulation without the use of any accessories. This is possible both for work with dry or oil immersion systems, and furthermore thanks to interference between the rays of light, individual preparations give a many-colored light.

IMPORTANCE OF THE SUPERCONDENSER 3D

Our condenser opens up new possibilities not only in the realm of biological and medical microscopic research but also in industry and technology, in the investigation of synthetic fibers, in geology and so on. With the aid of this condenser we have already been able to obtain significant morphological results, mainly in the field of hematological investigation.

Our condenser will show, for example, particles which in the Abbé field appear only in the form of points (for example the granules in the red blood corpuscles and the leucocytes, and also certain details of the structure of diatoms).

Our condenser completely excludes the haloes characteristic of phase contract images obtained by the method of Zernik. On the basis of our microphotographs we believe that all the results of investigations carried out by the use of the phase contrast principle require critical review.

Our microphotographs obtained by the use of the 3D condenser and immersion objectives demonstrate the possibility of obtaining a degree of magnification which is 2 or 3 times greater than that attainable with previous condenser systems. It is understandable that this considerably increases the instructive and demonstrational value of the microscope as well as its documentational importance.

SUMMARY

A new construction of a microscope condenser – supercondenser (3D) based on combined illumination has been offered. The condenser consists of three frontal lenses forming an aplanatic system with a numerical aperture of 1.25.

Supercondenser 3D possesses the following advantages:

1). It improves the resolving power of the microscope.

2). It eliminates optical distortions.

3). It forms a plastic image of a three-dimensional object.

4). It is simple in use and may be utilized with ordinary microscopes-both for dry or immersion objectives.

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED

[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiia	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiia	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Embriol.	Arkhiv Anatomii Gistologii i Embriologii	Archives of Anatomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiia	Biokhimiia	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiia Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Biull. Eksptl. Biol. i Med.	Biulleten Eksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Biull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Biulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Eksptl. Khirurg.	Eksperimentalnaia Khirurgiia	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiia i Toksikologiia	Pharmacology and Toxicology
Farmatsiia	Farmatsiia	Pharmacy
Fiziol. Rastenii	Fiziologiia Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenova Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariia	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiia Akademii Nauk SSSR, Seriia Biologicheskaia	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiia Tikhookeanskogo N. I. Instituta Rybnogo Khoziaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiia	Khirurgiia	Surgery
Klin. Med.	Klinicheskaia Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaia Parazitologiia i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaia Radiologiia	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiia	Mikrobiologiia	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhiat. i Psikhogig.	Nevropatologiia, Psikhiatriia i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediia, Travmatologiia i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriia	Pediatriia	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Endokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniia Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaia Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journals
Stomatologiia	Stomatologiia	Stomatology
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriia	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics

* LIBRARY OF CONGRESS TRANSLITERATION SYSTEM.

(continued)

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Abbreviation	Journal	Translation
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiia	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiia	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiia, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoiuz. Gidrobiol. Obshchestva	Trudy Vsesoiuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoiuz. Inst. Ekspt. Med.	Trudy Vsesoiuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiia	Urologiia	Urology
Uspekhi Biokhimiia	Uspekhi Biokhimiia	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiia	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriia Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universiteta, Seriia Biologii i Pochvovedeniia	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiia	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariia	Veterinariia	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniia	Voprosy Pitaniia	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaia Laboratoriia	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiia	Journal of General Biology
Zhur. Vysshei Nerv. Deiatel.	Zhurnal Vysshei Nervnoi Deiatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

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